

EXHIBIT B13

Part 2

Copy Produced by Plaintiffs Prior to Deposition

— Cell Lines

SKOV-3

A2780

TOV112D

ATCC

Sigma Aldrich, St. Louis, MO)

A kind gift from Gensheng Wu at Wayne State Univ.

EL-1/macrophages

ATCC,

Normal ovarian epithelial

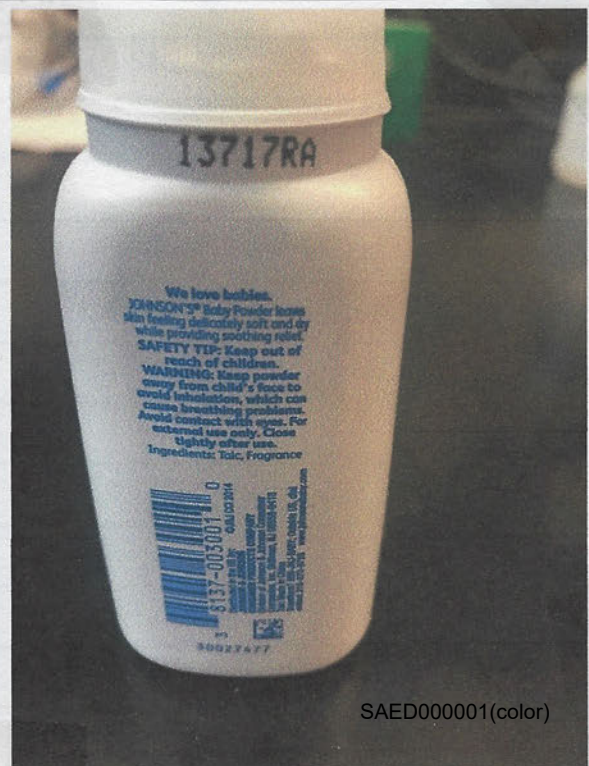
Cell Biologies, Chicago, IL

FT33

Applied Biological Materials, Richmond, BC, Canada

— Fetal bovine serum (FBS, Innovative Research, Novi, MI)
Penicillin/streptomycin (Fisher Scientific)

— Johnson Baby Powder (#30027578 Lot #13717RA)



SAED000001(color)

Seeded Cells for PCR

1/24/18

- Thawing Cells

EL-1 (Macrophages)

SKOV-3

TOU#12D

A2780

FT33

Normal Ovarian Epithelial

Media

IMDM (10% FBS, 1% PS, 1mL H-T)

McCoy's 5A (10% FBS, 1% PS)

Medium 199: MCDB 105 (1:1) + 10% FBS + 1% PS

RPMI-1640 (10% FBS + 1% PS)

DMEM (10% FBS, 1% PS)

Complete Human Epithelial Cell Medium kit
(Cell Biologics)75 cm² flask + 15 ml medium

1/26/18

- Subculture Cells

* Normal Ovarian Epithelial use trypsin from ScienCell

① Wash with PBS 10ml

② gently remove PBS

③ Pipet trypsin-EDTA 2ml onto the washed cells monolayer

④ 37°C incubator 1 ~ 5 minutes (SKOV-3 longer)

⑤ Check under microscope

⑥ Add fresh medium 8ml to inactivate trypsin, Then mix

⑦ Take 2ml to a new 100mm dish

⑧ Add 8ml fresh medium to 100mm dish

⑨ Incubate the cells

1/29/18

- Subculture Cells

2ml cells + 8ml medium 100 mm dish

Cells doubled in one day.

2/1/2018

- Subculture cells
- Seeded 1×10^6 cells 60mm dish + 5ml medium
- Need dose for treatment with talc
Unt, 5, 20, 100 ug/ml

Sample ID	
356	EL1 Unt
357	EL1 5 ug/ml Talc
358	EL1 20 ug/ml Talc
359	EL1 100 ug/ml Talc
360	SKOV-3 unt
361	SKOV-3 5ug/ml
362	SKOV-3 20ug/ml
363	SKOV-3 100ug/ml
364	TOV112 Unt
365	TOV112 5 ug/ml Talc
366	TOV112 20 ug/ml Talc
367	TOV112 100 ug/ml Talc
368	A2780 Unt
369	A2780 5 ug/ml
370	A2780 20 ug/ml
371	A2780 100 ug/ml
379	FT33 unt
380	FT33 5ug/ml
381	FT33 20 ug/ml
382	FT33 100 ug/ml
383	NOE unt
384	NOE 5 ug/ml Talc
385	NOE 20 ug/ml Talc
386	NOE 100 ug/ml Talc

2/2/2018

- treat cell with talc

prepare talc / Johnson Baby Powder (#30027578, lot 13717RA)

100mg talc + 10ml DMSO → mix $10\text{mg/ml} = 10^4\text{ug/ml}$

- Sterilization under UV light to avoid endotoxin and microbial contamination

- Powder 100mg suspended in DMSO and passed 5 times through 22-gauge needle and 0.2µl syringe filter

$$(X_1) (10^4\text{ug/ml}) = (5\text{ml}) (5\text{ug/ml}) \rightarrow X_1 = 2.5\text{ul}$$

$$(X_2) (10^4\text{ug/ml}) = (5\text{ml}) (20\text{ug/ml}) \rightarrow X_2 = 10\text{ul}$$

$$(X_3) (10^4\text{ug/ml}) = (5\text{ml}) (100\text{ug/ml}) \rightarrow X_3 = 50\text{ul}$$

2/5/2018

- Collect Cells (See below)

- RNA Extraction RNeasy Mini Kit (Qiagen cat #74106/go to Pg 33)

- Detect concentration of RNA by Nanodrop (go to Pg 35) (Thermo Fisher Scientific) (Life Technologies)

- cDNA Synthesis via Reverse Transcription - VILO Kit (go to Pg 33)

Put on gloves and spray with 70% ethanol

Remove cell culture dish from incubator

Observe cells under microscope.

Move the dishes to your work bench, does not need to be done in the hood.

Collect media and place in labeled 15ml tube for freezing.

Add 10 ml PBS

Using a cell scraper, scrape the bottom of the dish and rotate it to ensure scraping of entire bottom

Using a 10ml pipet, remove the PBS and cell mixture and place into the 15ml conical centrifugation tube that corresponds to the dish, 1ml for RNA, 2ml for DNA, 8 ml for protein assay.

Close and centrifuge all tubes, 5 minutes at 1800rpm (slower speed keeps cells from breaking).

Place another paper towel by sink, dump PBS from all tubes into sink and place tubes upside down to drain them. Cells will be collected at the bottom. Place all tubes in Styrofoam holder and place in -80°C freezer.

Cell Collection Protocol

RNA Extraction

RNeasy Mini Kit (Qiagen cat # 74106)

RNA ≈ 50 μl

Important Notes before starting: **WORK IN THE HOOD**

- **β-Mercaptoethanol (β-ME)** can be added to **Buffer RLT (lysis buffer)** before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 μl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for **one month** after addition of β-ME.
- **Buffer RPE** is supplied as a concentrate. Before using for the first time, add **ethanol** as indicated on the bottle. Be sure to **mark the lid with a X** to show that the working solution has been prepared.

Buffer RW1 and Buffer RLT are hazardous.

- Buffer RLT+ β-ME should be disposed of in the jar in the hood.
- Buffer RW1 should be disposed of in the jar in the hood.

Preparation of the Buffer RLT

- In a labeled 15ml centrifugation tube, add 10 μl β-ME for every 1 ml Buffer RLT.

Preparation of your samples

1. Add 350 μl of the **Buffer RLT + β-ME** solution to each of your sample tubes.
 - a. if you have a lot of cells, you will need to add 600 μl of Buffer RLT + β-ME solution to each tube
***also add equal volume of ethanol)
2. Add 350 μl of **70% ethanol** to each tube and pipet to mix
3. Transfer the entire sample to its corresponding mini spin column
 - a. Close columns and place them into the small centrifuge.
 - b. Centrifuge the tubes for 15 seconds at 13,000 rpm
4. Dump the flow through into hazardous waste jar **in the hood**.
5. Add 700 μl of the **Buffer RW1** to the RNeasy column
 - a. Centrifuge 15 seconds at 13,000 rpm
6. Dump the flow through into hazardous waste jar **in the hood**
7. Add 500 μl of **Buffer RPE** onto each RNeasy column
 - a. Centrifuge 15 seconds at 13,000 rpm
8. Dump the flow through into waste jar
9. Add 500 μl **Buffer RPE** to each column again
 - a. Centrifuge 2 minutes at 13,000 rpm to dry the silica gel membrane
10. Dump the flow through in waste jar, centrifuge for one minute more
11. Remove columns from collection tubes and place in corresponding 1.5ml centrifuge tube
12. Add 50 μl of **RNase-free water** to each column, onto the center of the silica-gel membrane without touching the sides of the column (water dissolves RNA).
 - a. Allow to stand for 1 minute
 - b. Centrifuge columns for 1 minute at 13,000 rpm, **LID MUST BE ON CENTRIFUGE**
13. Collect flow through from the collection tube and place back into the column on the center of the membrane, allow to stand for 1 minute
 - a. Centrifuge columns again for 1 minute at 13,000 rpm, **LID MUST BE ON CENTRIFUGE**
14. Remove and dispose of columns
15. Place your microcentrifuge tubes containing RNA on ice
 - a. Detect concentration of RNA
 - b. Good quality RNA has a A260/A280 of 2.0

Unit μg/μl

NEED TO MEASURE RNA EACH TIME YOU GO TO MAKE cDNA

cDNA Synthesis via Reverse Transcription - VILO kit

cDNA
20 µl

You will need:

Ice

Thaw, on ice:

RNA

VILO MasterMix

RNase-free water

You must detect the concentration of your RNA. After doing this, you can calculate the volume needed to get for a 1 µg reaction.

i.e. - If your RNA concentration is 0.9 ug/ul then:

$$(x \text{ ul})(0.9 \text{ ug/ul}) = 1 \text{ ug} \quad \text{solve for } x$$

For a single reaction, combine the following components in a sterile PCR tube on ice.

Component	1 µg RNA Volume/reaction
VILO MasterMix	4 µl
Template RNA	Variable up to 1 µg
RNase-free Water	Variable
Total Volume:	20 µl

The total amount in each tube should equal **20 µl**, hence the variable volume of water.

- Add 4 µl VILO MasterMix to each tube, volume of RNA calculated, volume of water calculated, and gently mix.
- Place the tubes in a rack and the rack into a 25°C water bath for 10 minutes.
- Place the rack into a 42°C water bath for 60 minutes.
- Then, place racked tubes into 85°C water bath for 5 minutes to terminate the reaction.
- Place samples on ice for a few minutes.
- Centrifuge cDNA.
- Place into -20°C freezer for storage or continue on to PCR.

RNA Concentration (Nanodrop)

Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample T
356	EL1 Unt	0.083	µg/µl	2.074	1.109	1.87	1.3	RNA
357	EL1 5 ug/ml Talc	0.1	µg/µl	2.5	1.342	1.86	1.18	RNA
358	EL1 20 ug/ml Talc	0.0829	µg/µl	2.073	1.118	1.85	1.26	RNA
359	EL1 100 ug/ml Talc	0.0349	µg/µl	0.873	0.476	1.84	0.39	RNA
360	SKOV-3 unt	0.2387	µg/µl	5.968	2.966	2.01	0.78	RNA
361	SKOV-3 5ug/ml	0.3389	µg/µl	8.473	4.194	2.02	1.15	RNA
362	SKOV-3 20ug/ml	0.3017	µg/µl	7.542	3.837	1.97	1.47	RNA
363	SKOV-3 100ug/ml	0.1118	µg/µl	2.796	1.465	1.91	1.53	RNA
364	TOV112 Unt	0.2401	µg/µl	6.003	2.879	2.09	1.78	RNA
365	TOV112 5 ug/ml Talc	0.2418	µg/µl	6.044	2.939	2.06	1.27	RNA
366	TOV112 20 ug/ml Talc	0.2043	µg/µl	5.106	2.459	2.08	1.88	RNA
367	TOV112 100 ug/ml Talc	0.1712	µg/µl	4.281	2.026	2.11	1.83	RNA
368	A2780 Unt	0.2203	µg/µl	5.508	2.88	1.91	1.34	RNA
369	A2780 5 ug/ml	0.2474	µg/µl	6.185	3.187	1.94	2.03	RNA
370	A2780 20 ug/ml	0.2217	µg/µl	5.541	2.855	1.94	1.63	RNA
371	A2780 100 ug/ml	0.1336	µg/µl	3.34	1.726	1.93	1.42	RNA
379	FT33 unt	0.1685	µg/µl	4.212	2.034	2.07	1.01	RNA
380	FT33 5ug/ml	0.0658	µg/µl	1.645	0.713	2.31	3.02	RNA
381	FT33 20 ug/ml	0.0801	µg/µl	2.003	0.891	2.25	0.96	RNA
382	FT33 100 ug/ml	0.3084	µg/µl	7.711	3.759	2.05	2.24	RNA
383	NOE unt	0.2921	µg/µl	7.303	3.582	2.04	1.09	RNA
384	NOE 5 ug/ml Talc	0.1812	µg/µl	4.531	2.179	2.08	2.15	RNA
385	NOE 20 ug/ml Talc	0.0869	µg/µl	2.172	0.971	2.24	1.31	RNA
386	NOE 100 ug/ml Talc	0.0816	µg/µl	2.289	1.126	2.03	1.43	RNA

0.5 ug RNA Reaction		
ID	ul RNA	ul water
356	6.0	10.0
357	5.0	11.0
358	6.0	10.0
359	14.3	1.7
360	2.1	13.9
361	1.5	14.5
362	1.7	14.3
363	4.5	11.5
364	2.1	13.9
365	2.1	13.9
366	2.4	13.6
367	2.9	13.1
368	2.3	13.7
369	2.0	14.0
370	2.3	13.7
371	3.7	12.3
379	3.0	13.0
380	7.6	8.4
381	6.2	9.8
382	1.6	14.4
383	1.7	14.3
384	2.8	13.2
385	5.8	10.2
386	6.3	9.7

0.5µg RNA was obtained from each sample following dilution as described by this table.

← cDNA (20µl) prepared

2/19/2018 qRT-PCR for β -actin

β -actin test — Standard

- Aliquot Standard

Standard come desiccated

Reconstitute with TE buffer.

Add TE buffer such that the concentration will be $100 \mu\text{M}$

↓ The volume of TE buffer is on the product sheet

Mix well

In a new 1.5ml microtube, add 5ul of standard to each tube

Put tubes into the concentrator machine for 20 minutes — Lids open

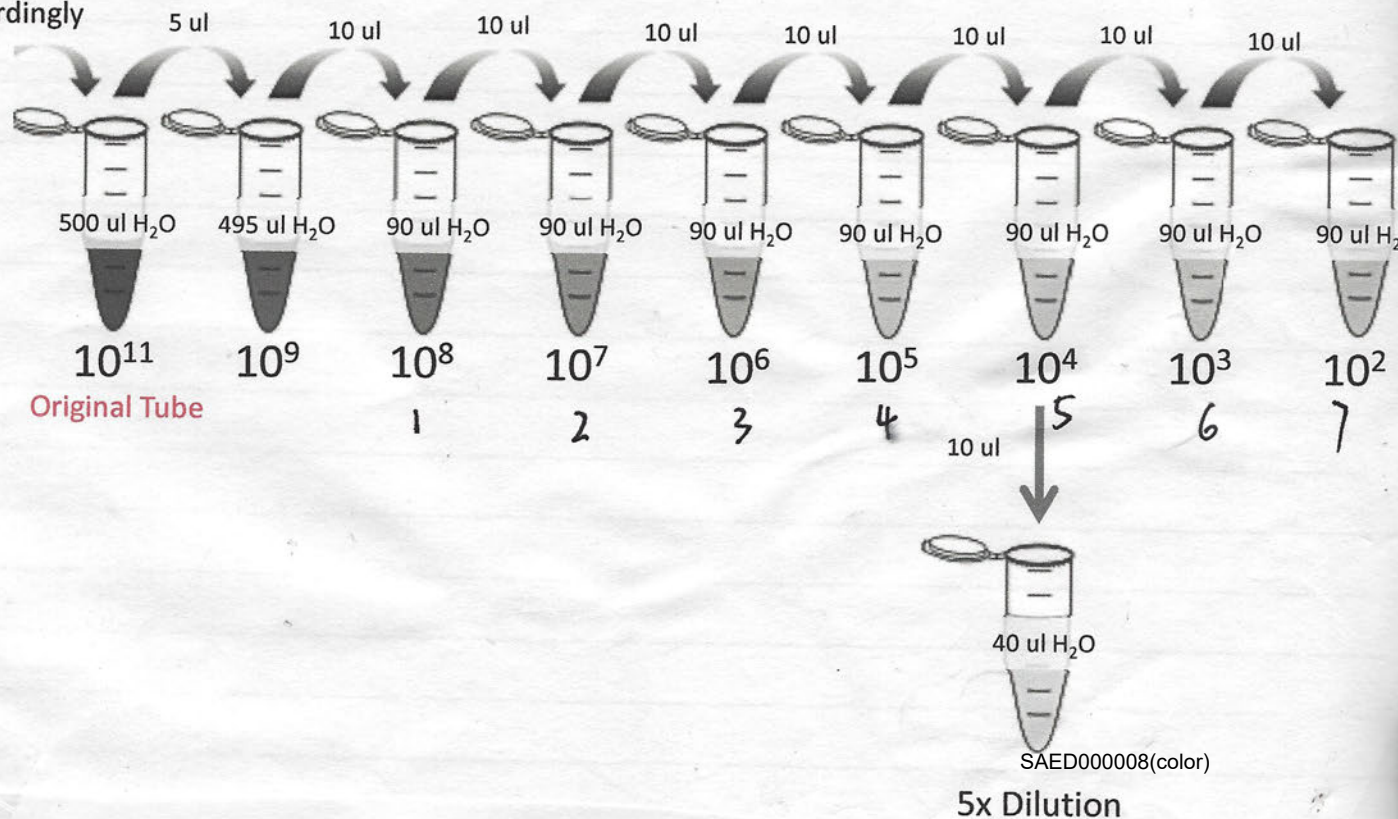
Then close tubes, and label

* Add 500ul PCR water to get a standard that is 10^{11}

Serial Dilution of Standard

Place samples on ice after mixing

Add amount of H_2O accordingly



2/19/2018

Run β -actin with samples 356 ~ 386

— Do 25 μ L reaction

	Water	9.5 μ L
→	Primer Forward	1 μ L
→	Primer Reverse	1 μ L
→	SYBR Green	12.5 μ L
→	Sample (cDNA)	1 μ L

20X dilution

(Radiant Green Lo-ROX qPCR Kit #Q5105)

— Calculating Mastermix for samples

$$72 \text{ samples} + 1 \text{ blank} = 73$$

$$73 \times 1.17 \text{ extra} = 85.41$$

— Mastermix calculation

$$\text{Water} = 9.5 \times 85.41 = 811.395 \approx 811.4 \mu\text{L}$$

$$\text{primer} = 1 \times 85.41 = 85.4 \mu\text{L}$$

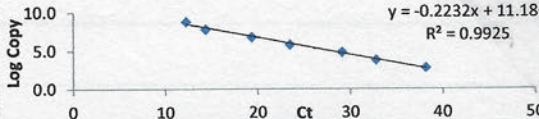
$$\text{SYBR green} = 12.5 \times 85.41 = 1067.625 = 1067.6 \mu\text{L}$$

— Mix then take 80.6 of this mix \rightarrow 1.5 ml tube / 1 per sample
 $73 \times 1.12 \text{ extra} = 80.6 \mu\text{L}$

— Add 3.4 μ L sample to 1.5 ml tube containing mastermix
 $3 \times 1.12 \text{ extra} = 3.4 \mu\text{L}$

— Mix well, add 25 μ L \rightarrow PCR tube.

3 total per sample

Run Summary (Smart Cycler 2.0d)					Log Copy				
In Name:	bactin 1ul 10x								
d Curve:	B-actin Standard RADIANT SYBR								
arted At:	2/19/2018 10:20								
umber of Sites:	72								
esults Table									
ite ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct	Melt Peak1	Y=Log Copy
8	64-10		8 STD		OK	610000000	12.22	79.62	8.8
9	64-10		7 STD		OK	610000000	14.33	79.86	7.8
-10			6 STD		OK	61000000	19.34	79.87	6.8
-11	64-10		5 STD		OK	6100000	23.46	79.84	5.8
-12	64-10		4 STD		OK	610000	29.09	79.92	4.8
-13	64-10		3 STD		OK	61000	32.81	80.08	3.8
-14	64-10		2 STD		OK	610	38.16	80.41	2.8
-1	b-Actin Radiant SYBR 20	356	UNKN		OK	285995.18	25.68	79.46	
-2	b-Actin Radiant SYBR 2017		UNKN		OK	273439.209	25.76	79.72	
-3	b-Actin Radiant SYBR 2017		UNKN		OK	409589.891	24.98	79.72	
-4	b-Actin Radiant SYBR 20	357	UNKN		OK	387206.6	25.09	79.77	
-5	b-Actin Radiant SYBR 2017		UNKN		OK	367150.863	25.19	79.65	
-6	b-Actin Radiant SYBR 2017		UNKN		OK	378655.448	25.13	79.78	
-7	b-Actin Radiant SYBR 20	358	UNKN		OK	230002.825	26.1	79.81	
-8	b-Actin Radiant SYBR 2017		UNKN		OK	274451.794	25.76	79.79	
-9	b-Actin Radiant SYBR 2017		UNKN		OK	204423.921	26.33	79.5	
-10	b-Actin Radiant SYBR 20	359	UNKN		OK	99410.671	27.73	79.7	
-11	b-Actin Radiant SYBR 2017		UNKN		OK	95098.867	27.82	79.69	
-12	b-Actin Radiant SYBR 2017		UNKN		OK	106962.324	27.59	79.93	
-13	b-Actin Radiant SYBR 20	360	UNKN		OK	82004.156	28.11	79.65	
-14	b-Actin Radiant SYBR 2017		UNKN		OK	76218.669	28.25	79.68	
-15	b-Actin Radiant SYBR 2017		UNKN		OK	80210.088	28.15	79.73	
-16	b-Actin Radiant SYBR 20	361	UNKN		OK	74149.095	28.3	79.69	
B1	b-Actin Radiant SYBR 2017		UNKN		OK	83584.072	28.07	79.9	
B2	b-Actin Radiant SYBR 2017		UNKN		OK	35471.637	29.74	79.66	
B3	b-Actin Radiant SYBR 20	362	UNKN		OK	67751.744	28.48	79.73	
B4	b-Actin Radiant SYBR 2017		UNKN		OK	81687.724	28.11	79.7	
B5	b-Actin Radiant SYBR 2017		UNKN		OK	100652.72	27.71	79.71	
B6	b-Actin Radiant SYBR 20	363	UNKN		OK	77232.773	28.22	79.88	
B7	b-Actin Radiant SYBR 2017		UNKN		OK	73843.031	28.31	79.65	
B8	b-Actin Radiant SYBR 2017		UNKN		OK	74279.769	28.3	79.85	
B9	b-Actin Radiant SYBR 20	364	UNKN		OK	78048.375	28.2	79.75	
B10	b-Actin Radiant SYBR 2017		UNKN		OK	75382.275	28.27	79.89	
B11	b-Actin Radiant SYBR 2017		UNKN		OK	67421.281	28.49	79.75	
B12	b-Actin Radiant SYBR 20	365	UNKN		OK	91845.321	27.89	79.95	
B13	b-Actin Radiant SYBR 2017		UNKN		OK	92266.631	27.88	79.7	
B14	b-Actin Radiant SYBR 2017		UNKN		OK	63374.184	28.61	79.77	
B15	b-Actin Radiant SYBR 20	366	UNKN		OK	41817.434	29.42	79.64	
B16	b-Actin Radiant SYBR 2017		UNKN		OK	49354.598	29.1	79.77	
C1	b-Actin Radiant SYBR 2017		UNKN		OK	65999.285	28.53	79.95	
C2	b-Actin Radiant SYBR 20	367	UNKN		OK	91668.153	27.89	79.92	
C3	b-Actin Radiant SYBR 2017		UNKN		OK	107294.783	27.58	79.68	
C4	b-Actin Radiant SYBR 2017		UNKN		OK	110651.012	27.52	79.89	
C5	b-Actin Radiant SYBR 20	368	UNKN		OK	89094.02	27.95	79.78	
C6	b-Actin Radiant SYBR 2017		UNKN		OK	77572.459	28.22	79.8	
C7	b-Actin Radiant SYBR 2017		UNKN		OK	106760.878	27.59	79.83	
C7	b-Actin Radiant SYBR 20	369	UNKN		OK	138914.317	27.08	79.87	
C8	b-Actin Radiant SYBR 2017		UNKN		OK	22379.944	30.63	79.83	
C9	b-Actin Radiant SYBR 2017		UNKN		OK	198224.635	26.39	79.72	
C11	b-Actin Radiant SYBR 20	370	UNKN		OK	132819.388	27.17	79.76	
C12	b-Actin Radiant SYBR 2017		UNKN		OK	100097.61	27.72	79.74	
C13	b-Actin Radiant SYBR 2017		UNKN		OK	46360.317	29.22	79.63	
C10	b-Actin Radiant SYBR 20	371	UNKN		OK	184842.26	26.52	79.63	
C11	b-Actin Radiant SYBR 2017		UNKN		OK	202714.758	26.35	79.72	
C12	b-Actin Radiant SYBR 2017		UNKN		OK	108192.324	27.57	79.81	
A1	b-Actin Radiant SYBR 20	379	UNKN		OK	307932.328	25.53	79.52	
A2	b-Actin Radiant SYBR 2017		UNKN		OK	377133.607	25.14	79.75	
A3	b-Actin Radiant SYBR 2017		UNKN		OK	542309.187	24.43	79.71	
A4	b-Actin Radiant SYBR 20	380	UNKN		OK	315038.876	25.49	79.88	
A5	b-Actin Radiant SYBR 2017		UNKN		OK	251730.241	25.92	79.59	
A6	b-Actin Radiant SYBR 2017		UNKN		OK	310158.171	25.52	79.58	
A7	b-Actin Radiant SYBR 20	381	UNKN		OK	328994.514	25.4	79.76	
A8	b-Actin Radiant SYBR 2017		UNKN		OK	298610.661	25.6	79.69	
A9	b-Actin Radiant SYBR 2017		UNKN		OK	271028.804	25.78	79.38	
A10	b-Actin Radiant SYBR 20	382	UNKN		OK	202182.58	26.35	79.57	
A11	b-Actin Radiant SYBR 2017		UNKN		OK	176291.01	26.62	79.73	
A12	b-Actin Radiant SYBR 2017		UNKN		OK	204290.69	26.33	79.76	
A13	b-Actin Radiant SYBR 20	383	UNKN		OK	188116.97	26.49	79.53	
A14	b-Actin Radiant SYBR 2017		UNKN		OK	176951.812	26.61	79.61	
A15	b-Actin Radiant SYBR 2017		UNKN		OK	185011.186	26.52	79.68	
A16	b-Actin Radiant SYBR 20	384	UNKN		OK	128937.209	27.23	79.69	
B1	b-Actin Radiant SYBR 2017		UNKN		OK	128406.079	27.23	79.78	
B2	b-Actin Radiant SYBR 2017		UNKN		OK	74621.79	28.29	79.68	
B3	b-Actin Radiant SYBR 20	385	UNKN		OK	115595.389	27.44	79.61	
B4	b-Actin Radiant SYBR 2017		UNKN		OK	160609.197	26.8	79.74	
B5	b-Actin Radiant SYBR 2017		UNKN		OK	172272.153	26.66	SAED000010 (color)	
B6	b-Actin Radiant SYBR 20	386	UNKN		OK	96396.19	27.79	79.9	
B7	b-Actin Radiant SYBR 2017		UNKN		OK	78347.732	28.2	79.71	
B8	b-Actin Radiant SYBR 2017		UNKN		OK	77149.198	28.23	79.78	

Case 3:16-md-02738-MAS-RLS Document 97-38-1 Filed 05/07/19 Page 13 of 48 PageID: 40884

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_001101	B-actin	ATGACTTAGTTGCGTTACACCTTTCTTGACAAACCTA ACTTGCGCAGAAACAGATGAGATTGGCATGGCTTTA TT	ATGACTTAGTTGCGTTAC	AATAAAGCCATGCCAATCTC	79	79	1220

Calculation data

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	10, 58	30, 72

Gene of Interest	B-actin	Unit	Formula											
Dalton - 1.66E-24 grams	1.66E-24	g												
Mass of base pair	615	Da												
avg. Mass/base	305.25	Da												
Length of entire gene	79	bases												
Mass in Daltons	2.41E+04	Da	- number bases x avg. mass/base											
Mass in grams	4.00E-20	g	- mass in Da x mass of a Da in grams											
Mass in ug	4.00E-14	ug	- above x 10E-6											
Mass in ng	4.00E-11	ng/copy	- above x 10E3											
/19/2018 10:20														
D	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA/ul cDNA	copies/ug RNA	Dilution Factor	Copies/ug RNA x Df	pg/ug RNA	Avg	Normalized avg	
356	EL1 Unt	285995.18	1	285995	0.5	20	0.025	1.14E+07	10	1.14E+08	4.58	4.48	1.03	
		273439.21	1	273439	0.5	20	0.025	1.09E+07	10	1.09E+08	4.38			
		409589.89	1	409590	0.5	20	0.025	1.64E+07	10	1.64E+08	6.56			
357	EL1 5 ug/ml Talc	287206.6	1	287207	0.5	20	0.025	1.15E+07	10	1.15E+08	4.60	4.45	1.02	
		267150.86	1	267151	0.5	20	0.025	1.07E+07	10	1.07E+08	4.28			
		278655.45	1	278655	0.5	20	0.025	1.11E+07	10	1.11E+08	4.46			
358	EL1 20 ug/ml Talc	230002.83	1	230003	0.5	20	0.025	9.20E+06	10	9.20E+07	3.68	3.48	0.80	
		274451.79	1	274452	0.5	20	0.025	1.10E+07	10	1.10E+08	4.39			
		204423.92	1	204424	0.5	20	0.025	8.18E+06	10	8.18E+07	3.27			
359	EL1 100 ug/ml Talc	294104.67	1	294105	0.5	20	0.025	1.18E+07	10	1.18E+08	4.71	4.36	1.00	
		250982.87	1	250983	0.5	20	0.025	1.00E+07	10	1.00E+08	4.02			
		246925.32	1	246925	0.5	20	0.025	9.88E+06	10	9.88E+07	3.95			
360	SKOV-3 Unt	82004.156	1	82004.2	0.5	20	0.025	3.28E+06	10	3.28E+07	1.31	1.27	1.06	
		76218.669	1	76218.7	0.5	20	0.025	3.05E+06	10	3.05E+07	1.22			
		80210.088	1	80210.1	0.5	20	0.025	3.21E+06	10	3.21E+07	1.28			
361	SKOV-3 5 ug/ml	74149.095	1	74149.1	0.5	20	0.025	2.97E+06	10	2.97E+07	1.19	1.26	1.05	
		83584.072	1	83584.1	0.5	20	0.025	3.34E+06	10	3.34E+07	1.34			
		35471.637	1	35471.6	0.5	20	0.025	1.42E+06	10	1.42E+07	0.57			
362	SKOV-3 20 ug/ml	67751.744	1	67751.7	0.5	20	0.025	2.71E+06	10	2.71E+07	1.08	1.33	1.11	
		81687.724	1	81687.7	0.5	20	0.025	3.27E+06	10	3.27E+07	1.31			
		100652.72	1	100653	0.5	20	0.025	4.03E+06	10	4.03E+07	1.61			
363	SKOV-3 100 ug/ml	77232.773	1	77232.8	0.5	20	0.025	3.09E+06	10	3.09E+07	1.24	1.20	1.00	
		73843.031	1	73843	0.5	20	0.025	2.95E+06	10	2.95E+07	1.18			
		74279.769	1	74279.8	0.5	20	0.025	2.97E+06	10	2.97E+07	1.19			
364	TOV112 Unt	78048.375	1	78048.4	0.5	20	0.025	3.12E+06	10	3.12E+07	1.25	1.24	1.69	
		75382.275	1	75382.3	0.5	20	0.025	3.02E+06	10	3.02E+07	1.21			
		67421.281	1	67421.3	0.5	20	0.025	2.70E+06	10	2.70E+07	1.08			
365	TOV112 5 ug/ml Talc	91845.321	1	91845.3	0.5	20	0.025	3.67E+06	10	3.67E+07	1.47	1.47	2.02	
		92266.631	1	92266.6	0.5	20	0.025	3.69E+06	10	3.69E+07	1.48			
		63374.184	1	63374.2	0.5	20	0.025	2.53E+06	10	2.53E+07	1.01			
366	TOV112 20 ug/ml Talc	41817.434	1	41817.4	0.5	20	0.025	1.67E+06	10	1.67E+07	0.67	0.73	1.00	
		49354.598	1	49354.6	0.5	20	0.025	1.97E+06	10	1.97E+07	0.79			
		65999.285	1	65999.3	0.5	20	0.025	2.64E+06	10	2.64E+07	1.06			
367	TOV112 100 ug/ml	91668.153	1	91668.2	0.5	20	0.025	3.67E+06	10	3.67E+07	1.47	1.74	2.39	
		107294.78	1	107295	0.5	20	0.025	4.29E+06	10	4.29E+07	1.72			
		110651.01	1	110651	0.5	20	0.025	4.43E+06	10	4.43E+07	1.77			
368	A2780 Unt	89094.02	1	89094	0.5	20	0.025	3.56E+06	10	3.56E+07	1.43	1.33	1.00	
		77572.459	1	77572.5	0.5	20	0.025	3.10E+06	10	3.10E+07	1.24			
		106760.88	1	106761	0.5	20	0.025	4.27E+06	10	4.27E+07	1.71			
369	A2780 5 ug/ml	138914.32	1	138914	0.5	20	0.025	5.56E+06	10	5.56E+07	2.22	2.70	2.02	
		22379.944	1	22379.9	0.5	20	0.025	8.95E+05	10	8.95E+06	0.36			
		198224.64	1	198225	0.5	20	0.025	7.93E+06	10	7.93E+07	3.17			
370	A2780 20 ug/ml	132819.39	1	132819	0.5	20	0.025	5.31E+06	10	5.31E+07	2.13	1.86	1.40	
		100097.61	1	100098	0.5	20	0.025	4.00E+06	10	4.00E+07	1.60			
		46360.317	1	46360.3	0.5	20	0.025	1.85E+06	10	1.85E+07	0.74			
391	A2780 100 ug/ml	184842.26	1	184842	0.5	20	0.025	7.39E+06	10	7.39E+07	2.96	3.10	2.33	
		202714.76	1	202715	0.5	20	0.025	8.11E+06	10	8.11E+07	3.25			
		108192.32	1	108192	0.5	20	0.025	4.33E+06	10	4.33E+07	1.73			
379	FT33 unt	307932.33	1	307932	0.5	20	0.025	1.23E+07	10	1.23E+08	4.93	5.48	1.26	
		377133.61	1	377134	0.5	20	0.025	1.51E+07	10	1.51E+08	6.04			
		542309.19	1	542309	0.5	20	0.025	2.17E+07	10	2.17E+08	8.68			
380	FT33 5ug/ml	315038.88	1	315039	0.5	20	0.025	1.26E+07	10	1.26E+08	5.04	4.54	1.04	
		251730.24	1	251730	0.5	20	0.025	1.01E+07	10	1.01E+08	4.03			
		310158.17	1	310158	0.5	20	0.025	1.24E+07	10	1.24E+08	4.97			
381	FT33 20ug/ml	328994.51	1	328995	0.5	20	0.025	1.32E+07	10	1.32E+08	5.27	4.54	1.04	
		296610.66	1	296611	0.5	20	0.025	1.19E+07	10	1.19E+08	4.75			
		271028.8	1	271029	0.5	20	0.025	1.08E+07	10	1.08E+08	4.34			
382	FT33 100 ug/ml	202182.58	1	202183	0.5	20	0.025	8.09E+06	10	8.09E+07	3.24	3.25	2.71	
		176291.01	1	176291	0.5	20	0.025	7.05E+06	10	7.05E+07	2.82			
		204290.69	1	204291	0.5	20	0.025	8.17E+06	10	8.17E+07	3.27			
383	NOE unt	188116.97	1	188117	0.5	20	0.025	7.52E+06	10	7.52E+07	3.01	2.94	2.44	
		176951.81	1	176952	0.5	20	0.025	7.08E+06	10	7.08E+07	2.83			
		185011.19	1	185011	0.5	20	0.025	7.40E+06	10	7.40E+07	2.96			
384	NOE 5ug/ml	128937.21	1	128937	0.5	20	0.025	5.16E+06	10	5.16E+07	2.06	2.06	1.71	
		128406.08	1	128406	0.5	20	0.025	5.14E+06	10	5.14E+07	2.06			
		74621.79	1	74621.8	0.5	20	0.025	2.98E+06	10	2.98E+07	1.19			
385	NOE 20ug/ml	115595.39	1	115595	0.5	20	0.025	4.62E+06	10	4.62E+07	1.85	2.67	2.00	
		160609.2	1	160609	0.5	20	0.025	6.42E+06	10	6.42E+07	2.57			
		172272.15	1	172272	0.5	20	0.025	6.89E+06	10	6.89E+07	2.76			
386	NOE 100 ug/ml	96396.19	1	96396.2	0.1856	20	0.00928	1.04E+07	10	1.04E+08	4.16	3.35	2.51	
		78347.732	1	78347.7	0.1856	20	0.00928	8.44E+06	10	8.44E+07	3.38			
		77149.198	1	77149.2	0.1856	20	0.00928	8.31E+06	10	8.31E+07	3.33			

SAED000011(color)

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Run qPCR CAT with samples 356 ~ 386

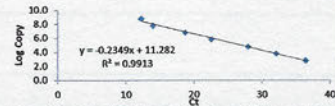
Primer info

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product / Amplicon Length	Start Position
NM_001852 Ordered Feb 2015	CAT	GGTTGAACAGATAGCCTTCGACCCAAAGCAACATGCCAC CTGGCATTGAGGCCAGTCTGACAAAATGCTTCAGGCG CGCCTTTTGCCTATCTGACACTACCG	GGTTGAACAGATAGCCTTC	CGGTGAGTGTCTCAGGATAG	105	105	1073

Raw data

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	10, 60	30, 72

Run Summary (Smart Cycler 2.0d)														
Run Name: CAT 356-370 Jul 10x														
Std Curve: CAT Standard RADIANT SYBR														
Started At: 2/19/2018 14:30														
Number of s: 52														
Results Table														
Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Re	FAM Ct	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Ct	Melt Peak1	Y=Log Copy
C15	67-10	8 STD	OK			606000000	12.2	0	16.91	0	0	0	85.46	8.8
C16	67-10	7 STD	OK			606000000	13.94	0	0	0	0	0	85.38	7.8
D1	67-10	6 STD	OK			6060000.5	18.73	0	23.83	0	0	0	85.46	6.8
D2	67-10	5 STD	OK			6060000	22.55	0	29.61	0	0	0	85.43	5.8
D3	67-10	4 STD	OK			606000	27.97	0	32.92	0	0	0	85.47	4.8
D4	67-10	3 STD	OK			60600	32.1	0	37.43	0	0	0	85.61	3.8
D5	67-10	2 STD	OK			6060	36.4	0	0	0	0	0	85.74	2.8
A2	CAT - RADIANT SYBR 2017	356 UNKN	OK			17855.978	29.94	17678.93714	0	ND	0	0	85.43	
A3	CAT - RADIANT SYBR 2017	UNKN	OK			16139.221	30.13	15950.97166	0	ND	0	0	85.56	
A4	CAT - RADIANT SYBR 2017	UNKN	OK			4915.959	32.32	4874.35504	0	ND	0	0	85.49	
A5	CAT - RADIANT SYBR 2017	357 UNKN	OK			12459.444	30.59	12400.50223	0	ND	0	0	85.36	
A6	CAT - RADIANT SYBR 2017	UNKN	OK			10272.547	30.96	10177.80459	0	ND	0	0	85.43	
A7	CAT - RADIANT SYBR 2017	UNKN	OK			14937.016	30.27	14786.76386	0	ND	0	0	85.33	
A8	CAT - RADIANT SYBR 2017	358 UNKN	OK			80205.3	31.41	7985.635205	0	ND	0	0	85.49	
A9	CAT - RADIANT SYBR 2017	UNKN	OK			8563.013	31.29	8521.143499	0	ND	0	0	85.19	
A10	CAT - RADIANT SYBR 2017	UNKN	OK			7784.922	31.46	7748.542868	0	ND	0	0	85.15	
A11	CAT - RADIANT SYBR 2017	359 UNKN	OK			5724.962	32.03	5695.992993	0	ND	0	0	85.3	
A12	CAT - RADIANT SYBR 2017	UNKN	OK			5383.508	32.15	5356.12657	0	ND	0	0	85.56	
A13	CAT - RADIANT SYBR 2017	UNKN	OK			4531.314	32.47	4494.20671	0	ND	0	0	85.51	
A14	CAT - RADIANT SYBR 2017	360 UNKN	OK			1178.499	30.79	11125.10317	0	ND	0	0	85.44	
A15	CAT - RADIANT SYBR 2017	UNKN	OK			1030.117	30.98	10081.30627	0	ND	0	0	85.47	
A16	CAT - RADIANT SYBR 2017	UNKN	OK			1112.669	30.97	10103.75917	0	ND	0	0	85.64	
B1	CAT - RADIANT SYBR 2017	361 UNKN	OK			1720.575	35.87	713.3668093	0	ND	0	0	85.69	
B2	CAT - RADIANT SYBR 2017	UNKN	OK			1536.288	36.42	529.6730491	0	ND	0	0	85.7	
B3	CAT - RADIANT SYBR 2017	UNKN	OK			1454.544	38.72	162.502786	0	ND	0	0	85.85	
B4	CAT - RADIANT SYBR 2017	362 UNKN	OK			988.97	35.29	976.4970234	0	ND	0	0	85.51	
B5	CAT - RADIANT SYBR 2017	UNKN	OK			892.134	36.24	583.8830707	0	ND	0	0	85.65	
B6	CAT - RADIANT SYBR 2017	UNKN	OK			743.008	37.24	339.8015296	0	ND	0	0	85.71	
B7	CAT - RADIANT SYBR 2017	363 UNKN	OK			930.122	35.40	920.0487999	0	ND	0	0	85.51	
B8	CAT - RADIANT SYBR 2017	UNKN	OK			891.701	35.48	881.0527584	0	ND	0	0	85.47	
B9	CAT - RADIANT SYBR 2017	UNKN	OK			917.542	38.60	162.736303	0	ND	0	0	85.56	
B10	CAT - RADIANT SYBR 2017	364 UNKN	OK			9774.808	31.04	9727.561426	0	ND	0	0	85.78	
B11	CAT - RADIANT SYBR 2017	UNKN	OK			8772.54	31.24	8729.735841	0	ND	0	0	85.51	
B12	CAT - RADIANT SYBR 2017	UNKN	OK			7079.596	31.64	7044.40926	0	ND	0	0	85.62	
B13	CAT - RADIANT SYBR 2017	365 UNKN	OK			6450.657	31.81	6418.341978	0	ND	0	0	85.52	
B14	CAT - RADIANT SYBR 2017	UNKN	OK			6345.112	31.84	6313.281374	0	ND	0	0	85.73	
B15	CAT - RADIANT SYBR 2017	UNKN	OK			6548.981	31.78	6516.215381	0	ND	0	0	85.96	
B16	CAT - RADIANT SYBR 2017	366 UNKN	OK			4356.817	32.54	4334.267044	0	ND	0	0	85.61	
C1	CAT - RADIANT SYBR 2017	UNKN	OK			4125.06	32.64	4103.614077	0	ND	0	0	85.67	
C2	CAT - RADIANT SYBR 2017	UNKN	OK			4205.28	32.60	4183.45132	0	ND	0	0	85.83	
C4	CAT - RADIANT SYBR 2017	357 UNKN	OK			470.667	30.59	12400.50223	0	ND	0	0	85.63	
C5	CAT - RADIANT SYBR 2017	UNKN	OK			289.742	30.96	10177.80459	0	ND	0	0	85.57	
C6	CAT - RADIANT SYBR 2017	UNKN	OK			832.75	30.27	14786.76386	0	ND	0	0	85.72	
C7	CAT - RADIANT SYBR 2017	368 UNKN	OK			13187.089	30.49	13125.02211	0	ND	0	0	85.75	
C8	CAT - RADIANT SYBR 2017	UNKN	OK			11210.87	30.79	11157.33328	0	ND	0	0	85.58	
C9	CAT - RADIANT SYBR 2017	UNKN	OK			12105.843	30.65	12048.42634	0	ND	0	0	85.78	
C10	CAT - RADIANT SYBR 2017	369 UNKN	OK			7785.982	31.46	7747.596091	0	ND	0	0	85.66	
C11	CAT - RADIANT SYBR 2017	UNKN	OK			9185.252	31.16	9140.612972	0	ND	0	0	85.58	
C12	CAT - RADIANT SYBR 2017	UNKN	OK			8973.241	31.20	8929.543543	0	ND	0	0	85.59	
C13	CAT - RADIANT SYBR 2017	370 UNKN	OK			6524.891	31.79	6492.236722	0	ND	0	0	85.61	
C14	CAT - RADIANT SYBR 2017	UNKN	OK			5247.982	32.19	5221.233205	0	ND	0	0	85.66	
C15	CAT - RADIANT SYBR 2017	371 UNKN	OK			5986.997	31.95	5956.815555	0	ND	0	0	85.66	



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Run qPCR CAT with samples 356 ~ 386

Primer info

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position	
NM_001852 Ordered Feb 2015	CAT	GGTTGAACAGATAGCCTTCGACCCAAGCAACATGCCAC CTGGCATTGAGGCCAGTCTGACAAATGCTTCAGGGC CGCCTTTTTTCGCTATCCTGACACTCACCG	GGTTGAACAGATAGCCTTC	CGGTGAGTGTGAGGATAG	105	105	1073	
					Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
					60	15	10, 60	30, 72

Raw data

Raw data

Run Summary (Smart Cycler 2.0d)														
Run Name: CAT 356-370 3ul 10x														
Std Curve: CAT Standard RADIANT SYBR														
Started At: 2/19/2018 14:30														
Number of: 52														
Results Table														
Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Re	FAM Ct	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Ct	Melt Peak1	Y=Log Copy
C15	67-10	8	STD		OK	606000000	12.2	0	16.91	0	0	0	85.45	8.8
C16	67-10	7	STD		OK	606000000	13.94	0	0	0	0	0	85.38	7.8
D1	67-10	6	STD		OK	6060000.5	18.73	0	23.83	0	0	0	85.46	6.8
D2	67-10	5	STD		OK	6060000	22.55	0	29.61	0	0	0	85.43	5.8
D3	67-10	4	STD		OK	606000	27.97	0	32.92	0	0	0	85.47	4.8
D4	67-10	3	STD		OK	60600	32.1	0	37.43	0	0	0	85.61	3.8
D5	67-10	2	STD		OK	606	36.4	0	0	0	0	0	85.74	2.8
A2	CAT - RADIANT SYBR 2017	356	UNKN		OK	17855.978	29.94	17678.93714	0	ND	0	0	85.43	
A3	CAT - RADIANT SYBR 2017		UNKN		OK	16139.221	30.13	15950.97166	0	ND	0	0	85.56	
A4	CAT - RADIANT SYBR 2017		UNKN		OK	4915.959	32.32	4874.36504	0	ND	0	0	85.49	
A5	CAT - RADIANT SYBR 2017	357	UNKN		OK	12459.444	30.59	12400.80223	0	ND	0	0	85.36	
A6	CAT - RADIANT SYBR 2017		UNKN		OK	10272.547	30.96	10177.80459	0	ND	0	0	85.43	
A7	CAT - RADIANT SYBR 2017		UNKN		OK	14937.016	30.27	14786.76386	0	ND	0	0	85.33	
A8	CAT - RADIANT SYBR 2017	358	UNKN		OK	80205.3	31.41	7985.839205	0	ND	0	0	85.49	
A9	CAT - RADIANT SYBR 2017		UNKN		OK	8583.013	31.29	8521.143499	0	ND	0	0	85.19	
A10	CAT - RADIANT SYBR 2017		UNKN		OK	7784.922	31.46	7746.542868	0	ND	0	0	85.15	
A11	CAT - RADIANT SYBR 2017	359	UNKN		OK	5724.962	32.03	5695.992993	0	ND	0	0	85.3	
A12	CAT - RADIANT SYBR 2017		UNKN		OK	5383.508	32.15	5356.12657	0	ND	0	0	85.56	
A13	CAT - RADIANT SYBR 2017		UNKN		OK	4531.314	32.47	4494.20671	0	ND	0	0	85.51	
A14	CAT - RADIANT SYBR 2017	360	UNKN		OK	1178.499	30.79	11125.10317	0	ND	0	0	85.44	
A15	CAT - RADIANT SYBR 2017		UNKN		OK	1030.117	30.98	10081.30627	0	ND	0	0	85.47	
A16	CAT - RADIANT SYBR 2017		UNKN		OK	1112.669	30.97	10103.75917	0	ND	0	0	85.64	
B1	CAT - RADIANT SYBR 2017	361	UNKN		OK	1720.575	35.87	713.3688093	0	ND	0	0	85.69	
B2	CAT - RADIANT SYBR 2017		UNKN		OK	1536.288	36.42	529.6730491	0	ND	0	0	85.7	
B3	CAT - RADIANT SYBR 2017		UNKN		OK	1454.544	38.72	152.502786	0	ND	0	0	85.65	
B4	CAT - RADIANT SYBR 2017	362	UNKN		OK	888.97	35.29	976.4970234	0	ND	0	0	85.51	
B5	CAT - RADIANT SYBR 2017		UNKN		OK	892.134	36.24	583.8830707	0	ND	0	0	85.65	
B6	CAT - RADIANT SYBR 2017		UNKN		OK	743.008	37.24	339.8019296	0	ND	0	0	85.71	
B7	CAT - RADIANT SYBR 2017	363	UNKN		OK	930.122	35.40	920.0467999	0	ND	0	0	85.51	
B8	CAT - RADIANT SYBR 2017		UNKN		OK	891.701	35.48	881.0527594	0	ND	0	0	85.47	
B9	CAT - RADIANT SYBR 2017		UNKN		OK	817.642	38.60	162.738303	0	ND	0	0	85.56	

Run Summary (Smart Cycler 2.0d)														
Run Name: CAT 3 ul of 10x 371-397														
Std Curve: CAT Standard RADIANT SYBR														
Started At: 2/19/2018 17:17														
Number of: 45														
Results Table														
Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Re	FAM Ct	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Ct	Melt Peak1	Y=Log Copy
C15	67-10	8	STD		OK	606000000	32.63	0	16.91	0	0	0	85.45	
C16	67-10	7	STD		OK	606000000	32.96	0	0	0	0	0	85.38	
D1	67-10	6	STD		OK	6060000.5	36.35	0	23.83	0	0	0	85.46	
D2	67-10	5	STD		OK	6060000	35.99	0	29.61	0	0	0	85.43	
D3	67-10	4	STD		OK	606000	36.00	0	32.92	0	0	0	85.47	
D4	67-10	3	STD		OK	60600	30.24	0	37.43	0	0	0	85.61	
D5	67-10	2	STD		OK	606	30.22	0	0	0	0	0	85.74	
A1	CAT - RADIANT SYBR 2017	371	UNKN		OK	1532.565	34.47	1523.954788	0	ND	0	0	85.27	
A2	CAT - RADIANT SYBR 2017		UNKN		OK	1236.764	34.86	1228.703382	0	ND	0	0	85.52	
A3	CAT - RADIANT SYBR 2017		UNKN		OK	1250.345	34.84	1243.212628	0	ND	0	0	85.7	
A4	CAT - RADIANT SYBR 2017	379	UNKN		OK	13751.972	31.09	9468.709467	0	ND	0	0	85.53	
A5	CAT - RADIANT SYBR 2017		UNKN		OK	14752.394	31.34	8281.755838	0	ND	0	0	85.48	
A6	CAT - RADIANT SYBR 2017		UNKN		OK	15528.954	31.49	7641.631079	0	ND	0	0	85.32	
A7	CAT - RADIANT SYBR 2017	380	UNKN		OK	12011.51	31.84	6312.993701	0	ND	0	0	85.46	
A8	CAT - RADIANT SYBR 2017		UNKN		OK	15456.092	31.86	6257.784843	0	ND	0	0	85.32	
A9	CAT - RADIANT SYBR 2017		UNKN		OK	14021.656	31.78	6523.18233	0	ND	0	0	85.15	
A10	CAT - RADIANT SYBR 2017	381	UNKN		OK	9278.117	32.52	4375.630618	0	ND	0	0	85.52	
A11	CAT - RADIANT SYBR 2017		UNKN		OK	6741.24	32.63	4123.440961	0	ND	0	0	85.4	
A12	CAT - RADIANT SYBR 2017		UNKN		OK	5417.343	32.56	4277.101171	0	ND	0	0	85.3	
A13	CAT - RADIANT SYBR 2017	382	UNKN		OK	11019.777	36.35	550.986046	0	ND	0	0	85.59	
A14	CAT - RADIANT SYBR 2017		UNKN		OK	10563.774	35.99	668.965621	0	ND	0	0	85.43	
A15	CAT - RADIANT SYBR 2017		UNKN		OK	9467.413	36.00	664.9291676	0	ND	0	0	85.46	
A16	CAT - RADIANT SYBR 2017	383	UNKN		OK	9763.023	30.24	15035.75313	0	ND	0	0	85.4	
B1	CAT - RADIANT SYBR 2017		UNKN		OK	8932.902	30.22	15218.38365	0	ND	0	0	85.7	
B2	CAT - RADIANT SYBR 2017		UNKN		OK	7723.593	30.20	15316.80267	0	ND	0	0	85.56	
B3	CAT - RADIANT SYBR 2017	384	UNKN		OK	4509.33	34.09	1866.845606	0	ND	0	0	85.66	
B4	CAT - RADIANT SYBR 2017		UNKN		OK	4792.009	34.18	1779.286131	0	ND	0	0	85.14	
B5	CAT - RADIANT SYBR 2017		UNKN		OK	3980.332	34.08	1876.663202	0	ND	0	0	85.61	
B6	CAT - RADIANT SYBR 2017	385	UNKN		OK	14691.73	35.48	883.7826164	0	ND	0	0	85.55	
B7	CAT - RADIANT SYBR 2017		UNKN		OK	13688.861	35.51	866.9833284	0	ND	0	0	85.36	
B8	CAT - RADIANT SYBR 2017		UNKN		OK	12783.958	35.64	807.210783	0	ND	0	0	85.7	
B9	CAT - RADIANT SYBR 2017	386	UNKN		OK	11571.379	35.98	673.8852469	0	ND	0	0	85.56	
B10	CAT - RADIANT SYBR 2017		UNKN		OK	11580.693	36.20	595.9832513	0	ND	0	0	85.66	
B11	CAT - RADIANT SYBR 2017		UNKN		OK	12700.554	36.13	619.2436347	0	ND	0	0	85.48	

SAE000013(color)

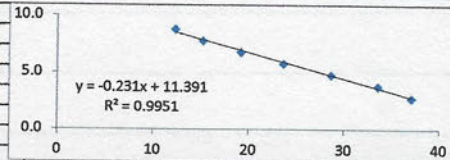
SAE D000013(color)

SAED000014(color)

2/20/2018

Run qPCR CSR with samples 356 ~ 368

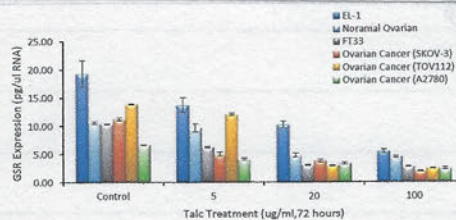
Run Summary (Smart Cycler 2.0d)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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primer information & calculation

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	10, 59	30, 72

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_000637	GSR	AAT CTC ACC AAG TCC CAT ATA GAA ATC ATC CGT GGC CAT AAG CCC ACA ATA GAG GTC AGT GGG AAA AAG TAC ACC GCC CCA CAC ATC CTG ATC GCC ACA GGT G	TCACCAAGTCCCATATAGAAATC	TGTGGCGATCAGGATGTG	103	116	



ID	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA x cDNA	copies/ug RNA	Dilution Factor	Copies/ug RNA x Df	pg/ug RNA	Normalized	Average	St Dev
356	EL1 Unt 72 hr	2491697.3	3	830565.78	0.5	20	0.025	3.32E+07	10	3.32E+08	17.34	16.89	19.25	2.360123
		3187855.3	3	1062618.4	0.5	20	0.025	4.25E+07	10	4.25E+08	22.18	21.62		
		6497579.9	3	2165860	0.5	20	0.025	8.66E+07	10	8.66E+08	45.22	44.06		
357	EL1 5 ug/ml Talc	2550060.2	3	850020.07	0.5	20	0.025	3.40E+07	10	3.40E+08	17.75	17.42	13.71	1.275139
		1465722	3	488574.01	0.5	20	0.025	1.95E+07	10	1.95E+08	10.20	10.01		
		2176688.1	3	725562.68	0.5	20	0.025	2.90E+07	10	2.90E+08	15.15	14.87		
358	EL1 20 ug/ml Talc	1215145.6	3	405048.52	0.5	20	0.025	1.62E+07	10	1.62E+08	8.46	10.61	10.19	0.58981
		1332012.2	3	444004.07	0.5	20	0.025	1.78E+07	10	1.78E+08	9.27	11.63		
		1119616	3	373205.33	0.5	20	0.025	1.49E+07	10	1.49E+08	7.79	9.78		
359	EL1 100 ug/ml Talc	747389.01	3	249129.67	0.5	20	0.025	9.97E+06	10	9.97E+07	5.20	5.20	5.43	0.324615
		674840.12	3	224946.71	0.5	20	0.025	9.00E+06	10	9.00E+07	4.70	4.70		
		813358.59	3	271119.53	0.5	20	0.025	1.08E+07	10	1.08E+08	5.66	5.66		
383	Normal Ovarian Unt 72 hr	1630605.2	3	543535.08	0.5	20	0.025	2.17E+07	10	2.17E+08	11.35	10.72	10.57	0.221873
		1582898.7	3	527632.9	0.5	20	0.025	2.11E+07	10	2.11E+08	11.02	10.41		
		1475190.9	3	491730.3	0.5	20	0.025	1.97E+07	10	1.97E+08	10.27	9.70		
384	5 ug/ml	1533631.1	3	511210.37	0.5	20	0.025	2.04E+07	10	2.04E+08	10.67	10.17	9.66	0.715034
		1381068.6	3	460356.2	0.5	20	0.025	1.84E+07	10	1.84E+08	9.61	9.15		
		994223.62	3	331407.87	0.5	20	0.025	1.33E+07	10	1.33E+08	6.92	6.59		
385	20 ug/ml	1232346.2	3	410782.07	0.5	20	0.025	1.64E+07	5	8.22E+07	4.29	3.86	4.79	0.333387
		1454072.8	3	484690.93	0.5	20	0.025	1.94E+07	5	9.69E+07	5.06	4.56		
		1604451.2	3	534817.08	0.5	20	0.025	2.14E+07	5	1.07E+08	5.58	5.03		
386	100 ug/ml	1313932.9	3	437977.64	0.5	20	0.025	1.75E+07	5	8.76E+07	4.57	4.57	4.45	0.173668
		1243346	3	414448.65	0.5	20	0.025	1.66E+07	5	8.29E+07	4.33	4.33		
		1120583.9	3	373527.98	0.5	20	0.025	1.49E+07	5	7.47E+07	3.90	3.90		
379	FT33 Unt 72 hr	832867.79	3	277622.6	0.5	20	0.025	1.11E+07	30	3.33E+08	17.39	10.27	10.31	0.052777
		904011.83	3	301337.28	0.5	20	0.025	1.21E+07	30	3.62E+08	18.87	11.15		
		838919.22	3	279639.74	0.5	20	0.025	1.12E+07	30	3.36E+08	17.51	10.35		
380	5 ug/ml Talc	911978.59	3	303992.86	0.5	20	0.025	1.22E+07	20	2.43E+08	12.69	6.29	6.21	0.104656
		890503.79	3	296834.6	0.5	20	0.025	1.19E+07	20	2.37E+08	12.39	6.14		
		924489.78	3	308163.26	0.5	20	0.025	1.23E+07	20	2.47E+08	12.87	6.37		
381	20 ug/ml Talc	892768.92	3	297589.64	0.5	20	0.025	1.19E+07	5	5.95E+07	3.11	3.11	3.05	0.084759
		903235.03	3	301078.34	0.5	20	0.025	1.20E+07	5	6.02E+07	3.14	3.14		
		858318.88	3	286106.29	0.5	20	0.025	1.14E+07	5	5.72E+07	2.99	2.99		
382	100 ug/ml Talc	937151.75	3	312383.92	0.5	20	0.025	1.25E+07	10	1.25E+08	6.52	2.73	2.72	0.059714
		914716.16	3	304905.39	0.5	20	0.025	1.22E+07	10	1.22E+08	6.37	2.66		
		955679.54	3	318559.85	0.5	20	0.025	1.27E+07	10	1.27E+08	6.65	2.78		
360	SKOV-3 Unt 72 hr	1630605.2	3	543535.08	0.5	20	0.025	2.17E+07	10	2.17E+08	11.35	11.35	11.18	0.234748
		1582898.7	3	527632.9	0.5	20	0.025	2.11E+07	10	2.11E+08	11.02	11.02		
		1475190.9	3	491730.3	0.5	20	0.025	1.97E+07	10	1.97E+08	10.27	10.27		
361	5 ug/ml	1533631.1	3	511210.37	0.5	20	0.025	2.04E+07	10	2.04E+08	10.67	5.28	5.01	0.371118
		1381068.6	3	460356.2	0.5	20	0.025	1.84E+07	10	1.84E+08	9.61	4.75		
		994223.62	3	331407.87	0.5	20	0.025	1.33E+07	10	1.33E+08	6.92	3.42		
362	20 ug/ml	1232346.2	3	410782.07	0.5	20	0.025	1.64E+07	5	8.22E+07	4.29	3.07	3.81	0.264745
		1454072.8	3	484690.93	0.5	20	0.025	1.94E+07	5	9.69E+07	5.06	3.62		
		1604451.2	3	534817.08	0.5	20	0.025	2.14E+07	5	1.07E+08	5.58	3.99		
363	100 ug/ml	1313932.9	3	437977.64	0.5	20	0.025	1.75E+07	5	8.76E+07	4.57	1.97	1.91	0.074685
		1243346	3	414448.65	0.5	20	0.025	1.66E+07	5	8.29E+07	4.33	1.86		
		1120583.9	3	373527.98	0.5	20	0.025	1.49E+07	5	7.47E+07	3.90	1.68		
364	TOV112 Unt 72 hr	832867.79	3	277622.6	0.5	20	0.025	1.11E+07	30	3.33E+08	17.39	13.83	13.88	0.071078
		904011.83	3	301337.28	0.5	20	0.025	1.21E+07	30	3.62E+08	18.87	15.02		
		838919.22	3	279639.74	0.5	20	0.025	1.12E+07	30	3.36E+08	17.51	13.94		
365	TOV112 5 ug/ml Talc	911978.59	3	303992.86	0.5	20	0.025	1.22E+07	20	2.43E+08	12.69	12.21	12.06	0.203256
		890503.79	3	296834.6	0.5	20	0.025	1.19E+07	20	2.37E+08	12.39	11.92		
		924489.78	3	308163.26	0.5	20	0.025	1.23E+07	20	2.47E+08	12.87	12.37		
366	TOV112 20 ug/ml Talc	892768.92	3	297589.64	0.5	20	0.025	1.19E+07	5	5.95E+07	3.11	2.98	2.93	0.081391
		903235.03	3	301078.34	0.5	20	0.025	1.20E+07	5	6.02E+07	3.14	3.02		
		858318.88	3	286106.29	0.5	20	0.025	1.14E+07	5	5.72E+07	2.99	2.87		
367	TOV112 100 ug/ml Talc	937151.75	3	312383.92	0.5	20	0.025	1.25E+07	10	1.25E+08	6.52	2.41	2.41	0.05276
		914716.16	3	304905.39	0.5	20	0.025	1.22E+07	10	1.22E+08	6.37	2.35		
		955679.54	3	318559.85	0.5	20	0.025	1.27E+07	10	1.27E+08	6.65	2.46		
368	A2780 Unt 72 hr	900868.73	3	302089.88	0.5	20	0.025	1.20E+07	10	1.20E+08	6.27	6.27	6.61	0.017274
		971821.88	3	323940.63	0.5	20	0.025	1.30E+07	10	1.30E+08	6.76	6.76		
		975332.42	3	325110.81	0.5	20	0.025	1.30E+07	10	1.30E+08	6.79	6.79		
369	5 ug/ml	391645.03	3	130548.34	0.5	20	0.025	5.22E+06	10	5.22E+07	2.73	1.59	4.07	0.21308
		1038721.6	3	346240.53	0.5	20	0.025	1.38E+07	10	1.38E+08	7.23	4.22		
		964544.98	3	321514.99	0.5	20	0.025	1.29E+07	10	1.29E+08	6.71	3.92		
370	20 ug/ml	1248521.6	3	416173.86	0.5	20	0.025	1.66E+07	10	1.66E+08	8.69	4.35	3.32	0.18132
		989162.63	3	329720.88	0.5	20	0.025	1.32E+07	10	1.32E+08	6.88	3.45		
		915563.77	3	305187.92	0.5	20	0.025	1.22E+07	10	1.22E+08	6.37	3.19		
371	100 ug/ml	922938.92	3	307646.31	0.5	20	0.025	1.23E+07	10	1.23E+08	6.42	2.56	2.40	0.21392
		813667.53	3	271222.51	0.5	20	0.025	1.08E+07	10	1.08E+08	5.66	2.25		
		1095341.3	3	365447.1	0.5	20	0.025	1.46E+07	10	1.46E+08	7.63	3.04		

SAED000016(color)

2/21/2018

Run PCR — iNOS with samples 356~368
 primer information

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position	
NM_000625	iNOS Dec	GAGGACCACATCTACCAGGAGGAGATGCTGGAGATGG CCCAGAAGGGGGTGCTGCATGCGGTGCACACAGCCTAT TCCGCGCTGCCTGG	GAGGACCACATCTACCAGGA	CCAGGCAGGCGGGAATAGC	89	89	3325	
					Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
					60	15	10,64	30, 72

Raw data

Run Summary (Smart Cycler 2.0d)															
Run Name: iNOS 3ul 10x															
Std Curve: new iNOS stand RAD 69-10 7to5x															
Started: 2/21/2018 12:10															
Number: 72															
Results Table															
Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/R	FAM Ct	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct	Melt Peak1	Y=Log Copy
C9	iNOS RADIANT SYBR 2017	7	STD		OK	61500000	12.38	0	15.89	0	0	0	0	86.47	7.8
C10	iNOS RADIANT SYBR 2017	6	STD		OK	6150000	14.94	0	18.64	0	0	0	0	86.2	6.8
C11	iNOS RADIANT SYBR 2017	5	STD		OK	615000	17.97	0	0	0	0	0	0	86.38	5.8
C12	iNOS RADIANT SYBR 2017	4	STD		OK	61500	22.07	0	25.97	0	0	0	0	86.37	4.8
C13	iNOS RADIANT SYBR 2017	3	STD		OK	6150	24.87	0	28.34	0	0	0	0	86.32	3.8
C14	iNOS RADIANT SYBR 2017	2	STD		OK	615	28.04	0	31.82	0	0	0	0	86.34	2.8
A1	iNOS RADIANT SYBR 2017	356	UNKN		OK	560.436	28.22	ND	31.19	ND	0	ND	0	85.98	
A2	iNOS RADIANT SYBR 2017		UNKN		OK	562.873	28.21	ND	31.95	ND	0	ND	0	86.25	
A3	iNOS RADIANT SYBR 2017		UNKN		OK	624.134	28.07	ND	0	ND	0	ND	0	86.29	
A4	iNOS RADIANT SYBR 2017	357	UNKN		OK	1763.75	26.61	ND	31.51	ND	0	ND	0	86.16	
A5	iNOS RADIANT SYBR 2017		UNKN		OK	1638.771	26.72	ND	32.64	ND	0	ND	0	86.24	
A6	iNOS RADIANT SYBR 2017		UNKN		OK	1605.089	26.75	ND	31.85	ND	0	ND	0	86.1	
A7	iNOS RADIANT SYBR 2017	358	UNKN		OK	2532.63	26.11	ND	33.61	ND	0	ND	0	86.31	
A8	iNOS RADIANT SYBR 2017		UNKN		OK	2620.276	26.06	ND	32.19	ND	0	ND	0	86.32	
A9	iNOS RADIANT SYBR 2017		UNKN		OK	2707.271	26.02	ND	32.13	ND	0	ND	0	85.84	
A10	iNOS RADIANT SYBR 2017	359	UNKN		OK	3441.472	25.68	ND	30.15	ND	0	ND	0	86.26	
A11	iNOS RADIANT SYBR 2017		UNKN		OK	3608.612	25.61	ND	30.53	ND	0	ND	0	86.28	
A12	iNOS RADIANT SYBR 2017		UNKN		OK	3779.663	25.55	ND	30.42	ND	0	ND	0	86.17	
A13	iNOS RADIANT SYBR 2017	360	UNKN		OK	1198.584	27.15	ND	33.97	ND	0	ND	0	86.28	
A14	iNOS RADIANT SYBR 2017		UNKN		OK	1212.664	27.14	ND	32.77	ND	0	ND	0	86.13	
A15	iNOS RADIANT SYBR 2017		UNKN		OK	1161.64	27.20	ND	33.97	ND	0	ND	0	86.03	
A16	iNOS RADIANT SYBR 2017	361	UNKN		OK	2173.64	26.32	ND	33.36	ND	0	ND	0	86.16	
B1	iNOS RADIANT SYBR 2017		UNKN		OK	2186.53	26.31	ND	0	ND	0	ND	0	86.35	
B2	iNOS RADIANT SYBR 2017		UNKN		OK	2121.38	26.36	ND	33.6	ND	0	ND	0	86.31	
B3	iNOS RADIANT SYBR 2017	362	UNKN		OK	3598.51	25.62	ND	0	ND	0	ND	0	86.25	
B4	iNOS RADIANT SYBR 2017		UNKN		OK	3480.36	25.67	ND	34.69	ND	0	ND	0	86.21	
B5	iNOS RADIANT SYBR 2017		UNKN		OK	3740.39	25.56	ND	34.54	ND	0	ND	0	86.31	
B6	iNOS RADIANT SYBR 2017	363	UNKN		OK	4066.248	25.45	ND	31.59	ND	0	ND	0	86.41	
B7	iNOS RADIANT SYBR 2017		UNKN		OK	4952.365	25.17	ND	31.94	ND	0	ND	0	86.17	
B8	iNOS RADIANT SYBR 2017		UNKN		OK	4028.142	25.46	ND	33.93	ND	0	ND	0	86.19	
B9	iNOS RADIANT SYBR 2017	364	UNKN		OK	994.502	27.42	ND	32.67	ND	0	ND	0	86.17	
B10	iNOS RADIANT SYBR 2017		UNKN		OK	942.888	27.49	ND	31.77	ND	0	ND	0	86.36	
B11	iNOS RADIANT SYBR 2017		UNKN		OK	1013.606	27.39	ND	32.72	ND	0	ND	0	86.2	
B12	iNOS RADIANT SYBR 2017	365	UNKN		OK	2450.062	26.16	ND	32.89	ND	0	ND	0	86.38	
B13	iNOS RADIANT SYBR 2017		UNKN		OK	21247.16	23.14	ND	31.75	ND	0	ND	0	86.28	
B14	iNOS RADIANT SYBR 2017		UNKN		OK	2644.139	26.05	ND	0	ND	0	ND	0	86.21	
B15	iNOS RADIANT SYBR 2017	366	UNKN		OK	2051.07	26.40	ND	31.11	ND	0	ND	0	86.2	
B16	iNOS RADIANT SYBR 2017		UNKN		OK	2061.872	26.40	ND	31.77	ND	0	ND	0	86.14	
C1	iNOS RADIANT SYBR 2017		UNKN		OK	2191.008	26.31	ND	31.62	ND	0	ND	0	86.33	
C2	iNOS RADIANT SYBR 2017	367	UNKN		OK	7818.086	24.53	ND	31.75	ND	0	ND	0	86.41	
C3	iNOS RADIANT SYBR 2017		UNKN		OK	7023.288	24.68	ND	31.65	ND	0	ND	0	86.28	
C4	iNOS RADIANT SYBR 2017		UNKN		OK	7233.577	24.64	ND	31.59	ND	0	ND	0	86.42	
C5	iNOS RADIANT SYBR 2017	368	UNKN		OK	833.854	27.66	ND	31.77	ND	0	ND	0	86.23	
C6	iNOS RADIANT SYBR 2017		UNKN		OK	980.02	27.44	ND	31.52	ND	0	ND	0	86.28	
C7	iNOS RADIANT SYBR 2017		UNKN		OK	907.709	27.54	ND	31.56	ND	0	ND	0	86.3	
C8	iNOS RADIANT SYBR 2017	369	UNKN		OK	2907.306	25.92	ND	32.85	ND	0	ND	0	86.21	
C9	iNOS RADIANT SYBR 2017		UNKN		OK	2721.779	26.01	ND	31.88	ND	0	ND	0	86.55	
C10	iNOS RADIANT SYBR 2017		UNKN		OK	2724.242	26.01	ND	31.74	ND	0	ND	0	86.3	
C11	iNOS RADIANT SYBR 2017	370	UNKN		OK	3991.927	25.47	ND	0	ND	0	ND	0	86.3	
C12	iNOS RADIANT SYBR 2017		UNKN		OK	3634.487	25.60	ND	32.38	ND	0	ND	0	86.25	
C13	iNOS RADIANT SYBR 2017		UNKN		OK	3748.078	25.56	ND	31.5	ND	0	ND	0	86.16	
C14	iNOS RADIANT SYBR 2017	371	UNKN		OK	8451.56	24.43	ND	33.8	ND	0	ND	0	86.29	
C15	iNOS RADIANT SYBR 2018		UNKN		OK	8687.2	24.39	ND	34.8	ND	1	ND	0	86.29	
C16	iNOS RADIANT SYBR 2019		UNKN		OK	8870.22	24.36	ND	35.8	ND	2	ND	0	86.29	
A4	iNOS RADIANT SYBR 2017	379	UNKN		OK	998.221	27.41	ND	31.39	ND	0	ND	0	86.11	
A5	iNOS RADIANT SYBR 2017		UNKN		OK	982.022	27.43	ND	32.63	ND	0	ND	0	86.01	
A6	iNOS RADIANT SYBR 2017		UNKN		OK	1011.299	27.39	ND	31.68	ND	0	ND	0	86	
A7	iNOS RADIANT SYBR 2017	380	UNKN		OK	2450.062	26.16	ND	31.59	ND	0	ND	0	86.28	
A8	iNOS RADIANT SYBR 2017		UNKN		OK	2324.16	26.23	ND	30.37	ND	0	ND	0	86.2	
A9	iNOS RADIANT SYBR 2017		UNKN		OK	2544.139	26.10	ND	30.53	ND	0	ND	0	85.85	
A10	iNOS RADIANT SYBR 2017	381	UNKN		OK	2131.035	26.35	ND	30.34	ND	0	ND	0	86.2	
A11	iNOS RADIANT SYBR 2017		UNKN		OK	2251.456	26.27	ND	30.29	ND	0	ND	0	86.09	
A12	iNOS RADIANT SYBR 2017		UNKN		OK	2161.65	26.33	ND	30.49	ND	0	ND	0	86.19	
A13	iNOS RADIANT SYBR 2017	382	UNKN		OK	7560.934	24.58	ND	33.65	ND	0	ND	0	86.13	
A14	iNOS RADIANT SYBR 2017		UNKN		OK	7752.091	24.55	ND	32.4	ND	0	ND	0	86.11	
A15	iNOS RADIANT SYBR 2017		UNKN		OK	7623.022	24.57	ND	31.81	ND	0	ND	0	86.2	
A16	iNOS RADIANT SYBR 2017	383	UNKN		OK	1044.584	27.35	ND	33.29	ND	0	ND	0	86.14	
B1	iNOS RADIANT SYBR 2017		UNKN		OK	1103.932	27.27	ND	0	ND	0	ND	0	86.31	
B2	iNOS RADIANT SYBR 2017		UNKN		OK	1115.921	27.25	ND	33.56	ND	0	ND	0	86.33	
B3	iNOS RADIANT SYBR 2017	384	UNKN		OK	1911.298	26.50	ND	0	ND	0	ND	0	86.32	
B4	iNOS RADIANT SYBR 2017		UNKN		OK	1872.882	26.53	ND	33.71	ND	0	ND	0	86.24	
B5	iNOS RADIANT SYBR 2017		UNKN		OK	1782.822	26.60	ND	33.58	ND	0	ND	0	86.32	
B6	iNOS RADIANT SYBR 2017	385	UNKN		OK	2780.221	25.98	ND	31.71	ND	0	ND	0	86.25	
B7	iNOS RADIANT SYBR 2017		UNKN		OK	2891.922	25.92	ND	32.63	ND	0	ND	0	86.15	
B8	iNOS RADIANT SYBR 2017		UNKN		OK	2777.119	25.98	ND	0	ND	0	ND	0	86.26	
B9	iNOS RADIANT SYBR 2017	386	UNKN		OK	4177.939	25.41	ND	32.58	ND	0	ND	0	86.38	
B10	iNOS RADIANT SYBR 2017		UNKN		OK	4522.782	25.30	ND	32.15	ND	0	ND	0	86.35	
B11	iNOS RADIANT SYBR 2017		UNKN		OK	4439.992	25.33	ND	32.68	ND	0	ND	0	86.07	

Lag Copy

Y = -0.3109x + 11.521

R² = 0.9966

Ct

SAED000017(color)

44

WEG 1044

Talc Treatment (μg/ml, 2 hours)	EL-1	Normal Ovarian	FT33	Ovarian Cancer (SKOV-3)	Ovarian Cancer (TOV112)
Control	~4.0	~5.0	~4.0	~6.0	~3.0
5	~10.0	~10.0	~8.0	~15.0	~10.0
20	~18.0	~18.0	~15.0	~15.0	~11.0
100	~21.0	~25.0	~18.0	~10.0	~20.0

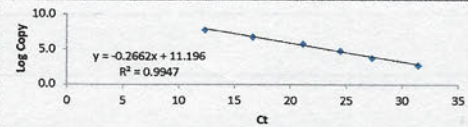
3/2/2018

Run PCR - MPO with samples

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product / Amplicon Length	Start Position
NM_000250	MPO Feb 2 A	CACTTGATCCTCTGGTTCTTCATTTATTGAGCACCTACT ACATGCAAGGCACGTACTAGGCGTGAGAAGCATATAG	CACTTGATCCTCTGGTTCTT	TCTATATGCTTCTCACGCCT	79	79	2859
					Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp
					60	15	63, 60
					extension time (s) and temp		
					30, 72		

Raw data

Run Summary (Smart Cycler 2.0d)											
Run Name:		MPO 3ul 10x taic									
Std Curve:		MPO test stand 60-60 new NK									
Started At:		3/2/2018 18:00									
Number of Sites:		72									
Site ID	Protocol	Sample ID	Sample Type	Status	FAM Std/Res	FAM Ct	Cy3 Std/Res	Cy3 Ct	Melt Peak1	Y=Log Copy	
B15	60 - 60	7	STD	OK	60900000	12.33	0	17.66	79.47	7.8	
B16	60 - 60	6	STD	OK	60900000	16.64	0	20.88	79.2	6.8	
C1	60 - 60	5	STD	OK	60900000	21.11	0	25.88	79.36	5.8	
C2	60 - 60	4	STD	OK	60900000	24.45	0	29.26	79.32	4.8	
C3	60 - 60	3	STD	OK	60900000	27.31	0	31	79.34	3.8	
C4	60 - 60	2	STD	OK	60900000	31.42	0	37.85	79.25	2.8	
C5	60 - 60	blank	blank	OK	60900000	31.42	0	37.85	79.25	2.8	
A1	MPO - RADIANT SYBR 2017	357	UNKN	OK	645.312	31.50	ND	31.34	79.29		
A2	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	416.587	32.22	ND	31.28	79.16		
A3	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	745.584	31.27	ND	0	79.24		
A4	MPO - RADIANT SYBR 2017	358	UNKN	OK	591.377	31.65	ND	31.1	79.24		
A5	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	552.923	31.76	ND	31.53	79.31		
A6	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	525.657	31.84	ND	30.98	79.32		
A7	MPO - RADIANT SYBR 2017	359	UNKN	OK	1796.114	29.83	ND	29.43	78.89		
A8	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	2128.677	29.56	ND	29.13	79.21		
A9	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	2217.772	29.49	ND	29.44	79.28		
A10	MPO - RADIANT SYBR 2017	361	UNKN	OK	29.258	36.55	ND	35.97	79.18		
A11	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	36.743	36.18	ND	36.3	79.28		
A12	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	36.982	36.17	ND	36.09	79.13		
A13	MPO - RADIANT SYBR 2017	362	UNKN	OK	82.652	34.86	ND	34.67	79.23		
A14	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	103.125	34.50	ND	33.27	79.16		
A15	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	107.922	34.42	ND	33.07	79.35		
A16	MPO - RADIANT SYBR 2017	363	UNKN	OK	342.972	32.53	ND	32.25	79.31		
B1	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	375.666	32.39	ND	0	79.25		
B2	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	214.756	33.30	ND	31.59	79.21		
B3	MPO - RADIANT SYBR 2017	365	UNKN	OK	301.332	32.75	ND	33.86	79.31		
B4	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	267.734	32.94	ND	32.93	79.41		
B5	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	283.642	32.84	ND	33.12	79.17		
B6	MPO - RADIANT SYBR 2017	366	UNKN	OK	319.869	32.65	ND	32.26	79.18		
B7	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	276.118	32.89	ND	32.33	79.17		
B8	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	282.458	32.85	ND	33.23	79.36		
B9	MPO - RADIANT SYBR 2017	367	UNKN	OK	948.423	30.88	ND	32.79	79.2		
B10	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	912.327	30.94	ND	32.57	79.12		
B11	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	924.748	30.92	ND	32.89	79.38		
B12	MPO - RADIANT SYBR 2017	368	UNKN	OK	218.768	33.27	ND	33.21	79.26		
B13	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	209.117	33.34	ND	32.98	79.21		
B14	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	164.816	33.73	ND	34.54	79.32		
B15	MPO - RADIANT SYBR 2017	370	UNKN	OK	214.533	33.30	ND	32.27	79.14		
B16	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	195.546	33.45	ND	33.29	79.41		
C1	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	212.351	33.32	ND	32.63	79.28		
C2	MPO - RADIANT SYBR 2017	371	UNKN	OK	805.293	31.14	ND	30.66	79.42		
C3	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	706.822	31.35	ND	30.85	79.23		
C4	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	853.694	31.05	ND	30.41	79.29		
C5	MPO - RADIANT SYBR 2017	379	UNKN	OK	669.3556	36.41	ND	31.09	79.3		
C6	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	664.4539	36.67	ND	30.96	79.21		
C7	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	669.0931	36.60	ND	30.87	79.12		
A1	MPO - RADIANT SYBR 2017	380	UNKN	OK	551.88	34.64	ND	28.49	78.82		
A2	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	327.683	34.69	ND	27.65	78.84		
A3	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	231.01065	34.67	ND	0	78.73		
A4	MPO - RADIANT SYBR 2017	381	UNKN	OK	12660.656	37.03	ND	27.51	78.77		
A5	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	11507.04	37.16	ND	28.56	78.53		
A6	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	10002.198	37.49	ND	28.15	78.7		
A7	MPO - RADIANT SYBR 2017	382	UNKN	OK	454.313	37.05	ND	33.23	79.04		
A8	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	434.4	37.21	ND	33.1	78.96		
A9	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	605.783	37.06	ND	33.12	78.78		
A10	MPO - RADIANT SYBR 2017	383	UNKN	OK	459.976	36.07	ND	33.23	78.91		
A11	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	270.276	36.18	ND	33.89	79.04		
A12	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	335.145	36.26	ND	33.59	79.21		
A13	MPO - RADIANT SYBR 2017	384	UNKN	OK	1506.613	38.20	ND	31.52	78.97		
A14	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	1446.257	38.28	ND	30.57	78.91		
A15	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	1187.594	38.16	ND	30.59	78.86		
A16	MPO - RADIANT SYBR 2017	385	UNKN	OK	1162.386	37.64	ND	31.26	79.02		
B1	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	886.67	37.37	ND	0	79.08		
B2	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	755.896	37.67	ND	31.5	79.12		
B3	MPO - RADIANT SYBR 2017	386	UNKN	OK	855.386	35.67	ND	33.71	78.97		
B4	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	981.45	35.79	ND	32.26	79.13		
B5	MPO - RADIANT SYBR 2017	UNKN	UNKN	OK	1212.295	35.72	ND	31.88	79.18		



Bar chart showing the effect of Talc treatment on the expression of genes related to ovarian cancer. The Y-axis represents Relative Expression (fold value) from 0.0000 to 2.5000. The X-axis shows Talc Treatment (µg/72 hours) with categories Control, 5, 20, and 100. The legend includes: MEF-1 (dark blue), Normal Ovarian (light blue), MTF33 (green), Ovarian Cancer (KOV3) (orange), Ovarian Cancer (TOV112) (yellow), and Ovarian Cancer (A2780) (red). Error bars represent standard deviation.

Talc Treatment (µg/72 hours)	MEF-1	Normal Ovarian	MTF33	Ovarian Cancer (KOV3)	Ovarian Cancer (TOV112)	Ovarian Cancer (A2780)
Control	~0.15	~0.10	~0.10	~0.10	~0.10	~0.10
5	~0.25	~0.15	~0.15	~0.15	~0.15	~0.15
20	~0.30	~0.20	~0.20	~0.20	~0.20	~0.20
100	~1.50	~0.20	~0.20	~2.00	~2.00	~2.00

3/2/2018

Run PCR - APX

Samples

Run Summary (Smart Cycler 2.0d)							
Run Name:	GPX Talc						
Std Curve:	GPX Standard RADIANT						
Started At:	3/2/2018 14:44						
Number of:	72						
Results Table							
Site ID	Protocol	Sample ID	Sample Type	Status	FAM Std/Res	FAM Ct	Melt Peak1
A1	GSTp1 - RADIANT SYBR 201	8	STD	OK	608000000	12.29	82.73
A2	GSTp1 - RADIANT SYBR 201	7	STD	OK	608000000	13.15	82.77
A3	GSTp1 - RADIANT SYBR 201	6	STD	OK	608000000	16.12	82.76
A4	GSTp1 - RADIANT SYBR 201	5	STD	OK	6080000	20.69	82.3
A5	GSTp1 - RADIANT SYBR 201	4	STD	OK	608000	24.74	82.72
A6	GSTp1 - RADIANT SYBR 201	3	STD	OK	6080	28.15	82.87
A7	GSTp1 - RADIANT SYBR 201	2	STD	OK	608	31.71	82.84
B1	GSTp1 - RADIANT SYBR 201	357	UNKN	OK	668201.923	20.84	82.11
B2	GSTp1 - RADIANT SYBR 2018		UNKN	OK	666753.61	20.84	82.95
B3	GSTp1 - RADIANT SYBR 2018		UNKN	OK	671705.856	20.83	82.07
B4	GSTp1 - RADIANT SYBR 201	358	UNKN	OK	206839.922	22.63	82.88
B5	GSTp1 - RADIANT SYBR 2018		UNKN	OK	230366.035	22.46	82.87
B6	GSTp1 - RADIANT SYBR 2018		UNKN	OK	210731.99	22.60	82.1
B7	GSTp1 - RADIANT SYBR 201	359	UNKN	OK	64785.937	24.40	82.1
B8	GSTp1 - RADIANT SYBR 2018		UNKN	OK	65867.594	24.38	82.75
B9	GSTp1 - RADIANT SYBR 2018		UNKN	OK	65675.403	24.38	82
B10	GSTp1 - RADIANT SYBR 201	361	UNKN	OK	508032.78	21.25	82
B11	GSTp1 - RADIANT SYBR 2018		UNKN	OK	479704.249	21.34	82.16
B12	GSTp1 - RADIANT SYBR 2018		UNKN	OK	488208.949	21.32	82.97
B13	GSTp1 - RADIANT SYBR 201	362	UNKN	OK	277671.948	22.18	82.91
B14	GSTp1 - RADIANT SYBR 2018		UNKN	OK	285591.813	22.14	82.03
B15	GSTp1 - RADIANT SYBR 2018		UNKN	OK	257832.145	22.29	82.9
B16	GSTp1 - RADIANT SYBR 201	363	UNKN	OK	195790.778	22.71	82.06
A1	GSTp1 - RADIANT SYBR 2018		UNKN	OK	174633.209	22.89	82.13
A2	GSTp1 - RADIANT SYBR 2018		UNKN	OK	193958.071	22.73	82.87
A3	GSTp1 - RADIANT SYBR 201	365	UNKN	OK	382147.473	21.69	82.17
A4	GSTp1 - RADIANT SYBR 2018		UNKN	OK	382528.579	21.69	82.13
A5	GSTp1 - RADIANT SYBR 2018		UNKN	OK	381507.876	21.69	82.15
A6	GSTp1 - RADIANT SYBR 201	366	UNKN	OK	165461.759	22.97	82.02
A7	GSTp1 - RADIANT SYBR 2018		UNKN	OK	142225.778	23.20	82.06
A8	GSTp1 - RADIANT SYBR 2018		UNKN	OK	148812.529	23.13	82.1
A9	GSTp1 - RADIANT SYBR 201	367	UNKN	OK	199402.777	22.68	82.04
A10	GSTp1 - RADIANT SYBR 2018		UNKN	OK	128707.691	23.35	82.91
A11	GSTp1 - RADIANT SYBR 2018		UNKN	OK	187371.231	22.78	82.29
A12	GSTp1 - RADIANT SYBR 201	369	UNKN	OK	454082.582	21.43	82.02
A13	GSTp1 - RADIANT SYBR 2018		UNKN	OK	411760.96	21.58	82.2
A14	GSTp1 - RADIANT SYBR 2018		UNKN	OK	439283.754	21.48	82.14
A15	GSTp1 - RADIANT SYBR 201	370	UNKN	OK	185507.125	22.80	82.17
A16	GSTp1 - RADIANT SYBR 2018		UNKN	OK	199908.926	22.68	82.1
C1	GSTp1 - RADIANT SYBR 2018		UNKN	OK	150814.91	23.11	82.19
C2	GSTp1 - RADIANT SYBR 201	371	UNKN	OK	90033.388	23.90	82.12
C3	GSTp1 - RADIANT SYBR 2018		UNKN	OK	92582.039	23.86	82.19
C4	GSTp1 - RADIANT SYBR 2018		UNKN	OK	77597.643	24.13	82.01
C5	GSTp1 - RADIANT SYBR 201	379	UNKN	OK	812750.693	22.97	82.21
C6	GSTp1 - RADIANT SYBR 2018		UNKN	OK	803430.814	23.20	82.21
C7	GSTp1 - RADIANT SYBR 2018		UNKN	OK	832511.564	23.13	82.79
B1	GSTp1 - RADIANT SYBR 201	380	UNKN	OK	600285.246	22.06	82.09
B2	GSTp1 - RADIANT SYBR 2018		UNKN	OK	562024.568	22.48	82.88
B3	GSTp1 - RADIANT SYBR 2018		UNKN	OK	1175903.995	22.13	82.04
B4	GSTp1 - RADIANT SYBR 201	381	UNKN	OK	718719.203	22.73	82.99
B5	GSTp1 - RADIANT SYBR 2018		UNKN	OK	680134.125	22.59	82.91
B6	GSTp1 - RADIANT SYBR 2018		UNKN	OK	572473.147	22.76	82.96
B7	GSTp1 - RADIANT SYBR 201	382	UNKN	OK	105507.125	23.66	82.96
B8	GSTp1 - RADIANT SYBR 2018		UNKN	OK	109908.926	23.60	82.55
B9	GSTp1 - RADIANT SYBR 2018		UNKN	OK	90980.998	23.89	82.94
B10	GSTp1 - RADIANT SYBR 201	383	UNKN	OK	1051419.196	21.25	82.98
B11	GSTp1 - RADIANT SYBR 2018		UNKN	OK	838587.722	21.34	82.03
B12	GSTp1 - RADIANT SYBR 2018		UNKN	OK	945596.546	21.32	82.02
B13	GSTp1 - RADIANT SYBR 201	384	UNKN	OK	800078.097	21.71	82.94
B14	GSTp1 - RADIANT SYBR 2018		UNKN	OK	851661.834	21.68	82.9
B15	GSTp1 - RADIANT SYBR 2018		UNKN	OK	706709.075	21.79	82.97
B16	GSTp1 - RADIANT SYBR 201	385	UNKN	OK	503377.481	22.97	82.17
A1	GSTp1 - RADIANT SYBR 2018		UNKN	OK	291290.52	22.89	82.15
A2	GSTp1 - RADIANT SYBR 2018		UNKN	OK	221345.011	22.73	82.95
A3	GSTp1 - RADIANT SYBR 201	386	UNKN	OK	333584.129	23.31	82.06
A4	GSTp1 - RADIANT SYBR 2018		UNKN	OK	344466.658	23.31	82.19
A5	GSTp1 - RADIANT SYBR 2018		UNKN	OK	348884.247	23.32	82.14
A7	GSTp1 - RADIANT SYBR 202	364	UNKN	OK	238896.626	22.41	82.2
A8	GSTp1 - RADIANT SYBR 2021		UNKN	OK	189277.851	22.76	82.09
A9	GSTp1 - RADIANT SYBR 2022		UNKN	OK	246310.592	22.36	83.18
A10	GSTp1 - RADIANT SYBR 202	360	UNKN	OK	981714.39	20.25	82.16
A11	GSTp1 - RADIANT SYBR 2024		UNKN	OK	970207.832	20.26	82.09
A12	GSTp1 - RADIANT SYBR 2025		UNKN	OK	1098811.105	20.07	83.34
A13	GSTp1 - RADIANT SYBR 202	356	UNKN	OK	812750.693	20.54	82.09
A14	GSTp1 - RADIANT SYBR 2027		UNKN	OK	803430.814	20.55	82.52
A15	GSTp1 - RADIANT SYBR 2028		UNKN	OK	832511.564	20.50	82.11
A16	GSTp1 - RADIANT SYBR 202	368	UNKN	OK	503377.481	21.27	82.91
A17	GSTp1 - RADIANT SYBR 2030		UNKN	OK	291290.52	22.11	82.12
A18	GSTp1 - RADIANT SYBR 2031		UNKN	OK	221345.011	22.53	83.12

10.0

5.0

0.0

0 5 10 15 20 25 30 35 40

$y = -0.231x + 11.391$

$R^2 = 0.9951$

SAED000021(color)

48

40895								
Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position	
NM_000581	GPX	GGACTACACCCAGATGAACGAGCTGCAGCGGCGCCT CGGACCCCGGGGCTGGTGGTGCTCGGCTTCCCGTG CAACAGTTTGGGCATCAGGAGAA	GGACTACACCCAGATGAAC	TTCTCTGATGCCCAAAC	100	100	242	
					Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
					60	15	10, 60	30, 72

Primer information & calculation

Primer information & calculation

Gene of Interest		GPX	Unit	Formula																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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3/2/2018

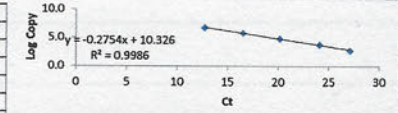
Run PCR - SOD3 with samples 356 ~ 386

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product / Amplicon Length
NM_000636	SOD3	GCGGTAGCACCAGCACTAGCAGCATGTTGAGCCGGG CAGTGTGCGGCACAGCAGGCAGCTGGCTCCGGTTT TGGGGTATCTGGGCTCC	GCGGTAGCACCAGCACTA	GGAGCCCAGATACCCCAA	85	85

Start Position	Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
132	60	15	10, 60	30, 72

Primer information

Run Summary (Smart Cycler 2.0d)											
Run Name:	SOD 3ul 10x taic										
Std Curve:	SOD test stand 60-60 new NK										
Started At:	3/2/2018 18:00										
Number of Sites:	72										



Results Table	Protocol	Sample ID	Sample Type	Status	FAM Std/Res	FAM Ct	Cy3 Std/Res	Cy3 Ct	Melt Peak1	log copy#
B15	60 - 60	8	STD	OK	610000000	0	0	0		86.47
B16	60 - 60	7	STD	OK	670000000	0	0	0		86.2
C1	60 - 60	6	STD	OK	6099999.5	12.75	0	16.88		86.36
C2	60 - 60	5	STD	OK	610000	16.52	0	20.46		86.37
C3	60 - 60	4	STD	OK	61000	20.15	0	23.88		86.32
C4	60 - 60	3	STD	OK	6100	24.06	0	27.62		86.34
C5	60 - 60	2	STD	OK	610	27.11	0	30.8		85.98
	SOD - RADIANT SYBR 2017	356	UNKN	OK	7693.556	23.38	0	31.34		85.98
	SOD - RADIANT SYBR 2018		UNKN	OK	7644.539	23.39	0	31.34		85.98
	SOD - RADIANT SYBR 2019		UNKN	OK	7690.931	23.38	0	31.34		85.98
A1	SOD - RADIANT SYBR 2020	357	UNKN	OK	6645.312	23.61	ND	31.34		86.25
A2	SOD - RADIANT SYBR 2017		UNKN	OK	6416.587	23.67	ND	31.28		86.29
A3	SOD - RADIANT SYBR 2017		UNKN	OK	6745.584	23.59	ND	0		86.16
A4	SOD - RADIANT SYBR 2017	358	UNKN	OK	2591.377	25.10	ND	31.1		86.24
A5	SOD - RADIANT SYBR 2017		UNKN	OK	2552.923	25.12	ND	31.53		86.1
A6	SOD - RADIANT SYBR 2017		UNKN	OK	2525.657	25.14	ND	30.98		86.31
A7	SOD - RADIANT SYBR 2017	359	UNKN	OK	796.114	26.96	ND	29.43		86.32
A8	SOD - RADIANT SYBR 2017		UNKN	OK	728.677	27.10	ND	29.13		85.84
A9	SOD - RADIANT SYBR 2017		UNKN	OK	717.772	27.12	ND	29.44		86.26
A10	SOD - RADIANT SYBR 2018	360	UNKN	OK	1454.313	26.01	ND	30.44		86.26
A11	SOD - RADIANT SYBR 2019		UNKN	OK	1434.403	26.03	ND	31.44		86.26
A12	SOD - RADIANT SYBR 2020		UNKN	OK	1605.783	25.85	ND	32.44		86.26
A10	SOD - RADIANT SYBR 2017	361	UNKN	OK	1239.258	26.26	ND	35.97		86.28
A11	SOD - RADIANT SYBR 2017		UNKN	OK	1316.749	26.17	ND	36.3		86.17
A12	SOD - RADIANT SYBR 2017		UNKN	OK	1368.982	26.11	ND	36.09		86.28
A13	SOD - RADIANT SYBR 2017	362	UNKN	OK	826.5135	26.90	ND	34.67		86.13
A14	SOD - RADIANT SYBR 2017		UNKN	OK	1038.125	26.54	ND	33.27		86.03
A15	SOD - RADIANT SYBR 2017		UNKN	OK	1071.519	26.49	ND	33.07		86.16
A16	SOD - RADIANT SYBR 2017	363	UNKN	OK	342.972	28.29	ND	32.25		86.35
B1	SOD - RADIANT SYBR 2017		UNKN	OK	375.666	28.15	ND	0		86.31
B2	SOD - RADIANT SYBR 2017		UNKN	OK	214.756	29.03	ND	31.59		86.25
B3	SOD - RADIANT SYBR 2018	364	UNKN	OK	2305.857	25.28	ND	32.59		86.25
B4	SOD - RADIANT SYBR 2019		UNKN	OK	2549.829	25.13	ND	33.59		86.25
B5	SOD - RADIANT SYBR 2020		UNKN	OK	2986.582	24.88	ND	34.59		86.25
B3	SOD - RADIANT SYBR 2017	365	UNKN	OK	1801.332	25.67	ND	33.86		86.21
B4	SOD - RADIANT SYBR 2017		UNKN	OK	1967.734	25.53	ND	32.93		86.31
B5	SOD - RADIANT SYBR 2017		UNKN	OK	1983.642	25.52	ND	33.12		86.41
B6	SOD - RADIANT SYBR 2017	366	UNKN	OK	931.869	26.71	ND	32.26		86.17
B7	SOD - RADIANT SYBR 2017		UNKN	OK	727.118	27.10	ND	32.33		86.19
B8	SOD - RADIANT SYBR 2017		UNKN	OK	828.458	26.90	ND	33.23		86.17
B9	SOD - RADIANT SYBR 2017	367	UNKN	OK	348.423	28.26	ND	32.79		86.36
B10	SOD - RADIANT SYBR 2017		UNKN	OK	312.327	28.44	ND	32.57		86.2
B11	SOD - RADIANT SYBR 2017		UNKN	OK	324.748	28.37	ND	32.89		86.38
B12	SOD - RADIANT SYBR 2018	368	UNKN	OK	324.748	28.38	ND	33.89		86.38
B13	SOD - RADIANT SYBR 2019		UNKN	OK	324.748	28.38	ND	34.89		86.38
B14	SOD - RADIANT SYBR 2020		UNKN	OK	324.748	28.38	ND	35.89		86.38
B12	SOD - RADIANT SYBR 2017	369	UNKN	OK	2218.768	25.34	ND	33.21		86.26
B13	SOD - RADIANT SYBR 2017		UNKN	OK	2209.117	25.35	ND	32.98		86.21
B14	SOD - RADIANT SYBR 2017		UNKN	OK	2388.2943	25.23	ND	34.54		86.2
B15	SOD - RADIANT SYBR 2017	370	UNKN	OK	1014.533	26.58	ND	32.27		86.14
B16	SOD - RADIANT SYBR 2017		UNKN	OK	1095.239	26.46	ND	33.29		86.33
C1	SOD - RADIANT SYBR 2017		UNKN	OK	1112.351	26.43	ND	32.63		86.41
C2	SOD - RADIANT SYBR 2017	371	UNKN	OK	805.293	26.94	ND	30.66		86.28
C3	SOD - RADIANT SYBR 2017		UNKN	OK	706.822	27.15	ND	30.85		86.42
C4	SOD - RADIANT SYBR 2017		UNKN	OK	853.694	26.85	ND	30.41		86.23
C5	SOD - RADIANT SYBR 2017	379	UNKN	OK	7693.556	25.09	ND	31.09		86.29
C6	SOD - RADIANT SYBR 2017		UNKN	OK	7644.539	24.94	ND	30.96		86.3
C7	SOD - RADIANT SYBR 2017		UNKN	OK	7690.931	24.91	ND	30.87		86.21
A1	SOD - RADIANT SYBR 2017	380	UNKN	OK	7155.188	25.76	ND	28.49		86.55
A2	SOD - RADIANT SYBR 2017		UNKN	OK	7276.83	25.62	ND	27.65		86.23
A3	SOD - RADIANT SYBR 2017		UNKN	OK	7101.065	25.54	ND	0		86.45
A4	SOD - RADIANT SYBR 2017	381	UNKN	OK	12660.656	26.81	ND	27.51		86.26
A5	SOD - RADIANT SYBR 2017		UNKN	OK	11507.04	27.14	ND	28.66		86.15
A6	SOD - RADIANT SYBR 2017		UNKN	OK	10002.198	26.93	ND	28.15		86.33
A7	SOD - RADIANT SYBR 2017	382	UNKN	OK	454.313	28.23	ND	33.23		86.2
A8	SOD - RADIANT SYBR 2017		UNKN	OK	434.4	28.34	ND	33.1		86.33
A9	SOD - RADIANT SYBR 2017		UNKN	OK	605.783	28.23	ND	33.12		86.55
A10	SOD - RADIANT SYBR 2017	383	UNKN	OK	459.976	25.91	ND	33.23		86.29
A11	SOD - RADIANT SYBR 2017		UNKN	OK	270.276	26.03	ND	33.89		86.34
A12	SOD - RADIANT SYBR 2017		UNKN	OK	335.145	25.91	ND	33.59		86.43
A13	SOD - RADIANT SYBR 2017	384	UNKN	OK	1508.613	26.28	ND	31.52		86.51
A14	SOD - RADIANT SYBR 2017		UNKN	OK	1446.257	26.16	ND	30.57		86.2
A15	SOD - RADIANT SYBR 2017		UNKN	OK	1187.594	26.11	ND	30.59		86.19
A16	SOD - RADIANT SYBR 2017	385	UNKN	OK	3162.386	26.72	ND	31.26		86.55
B1	SOD - RADIANT SYBR 2017		UNKN	OK	2886.67	26.59	ND	0		86.25
B2	SOD - RADIANT SYBR 2017		UNKN	OK	2755.896	26.58	ND	31.5		86.22
B3	SOD - RADIANT SYBR 2017	386	UNKN	OK	2855.386	28.08	ND	33.71		86.34
B4	SOD - RADIANT SYBR 2017		UNKN	OK	2981.45	28.12	ND	32.26		86.19
B5	SOD - RADIANT SYBR 2017		UNKN	OK	3212.295	27.99	ND	31.88		86.47

Bar chart showing SOD1 Expression (pg/ml RNA) for Control, 5, and 100 µg/ml Talc treatment at 72 hours. The chart compares four groups: EL-1 (blue), Normal Ovarian (light blue), FTS3 (dark blue), and Ovarian Cancer (SKOV-3) (red) and Ovarian Cancer (TOV112) (green). SOD1 expression is highest in the Control group and decreases with increasing Talc treatment concentration.

Talc Treatment (µg/ml, 72 hours)	EL-1	Normal Ovarian	FTS3	Ovarian Cancer (SKOV-3)	Ovarian Cancer (TOV112)
Control	~42,000	~10,000	~10,000	~10,000	~10,000
5	~38,000	~10,000	~10,000	~10,000	~10,000
100	~18,000	~10,000	~10,000	~10,000	~10,000

1/7/2018

protein extraction

Samples 356 ~ 386

ELISA

— Cells were seeded on 1-3-18 at a density of 1.2×10^6 cells per 150mm dish

— treat with talc ($10 \text{ mg/ml} = 10^4 \text{ } \mu\text{g/ml}$) \leftarrow 1-4-18
100mg talc + 10ml DMSO \rightarrow mix

Johnson & Johnson, #30027477, Lot# 13717RA)

$$\begin{aligned} (x_1) \cdot (10^4 \text{ } \mu\text{g/ml}) &= (5 \text{ ml}) (5 \text{ } \mu\text{g/ml}) &\rightarrow x_1 &= 2.5 \text{ ml} \\ (x_2) \cdot (10^4 \text{ } \mu\text{g/ml}) &= (5 \text{ ml}) (20 \text{ } \mu\text{g/ml}) &\rightarrow x_2 &= 10 \text{ } \mu\text{l} \\ (x_3) \cdot (10^4 \text{ } \mu\text{g/ml}) &= (5 \text{ ml}) (100 \text{ } \mu\text{g/ml}) &\rightarrow x_3 &= 50 \text{ } \mu\text{l} \end{aligned}$$

- after 72 hours treatment, collect cells and medium for ELISA
- Collect media and place in labeled 15ml tube for freezing
 - Then add 10ml PBS
 - Using cell scrape, scrape the bottom of the dish and rotate
 - Remove the PBS and cell mixture and place into 15ml labeled tubes
 - Centrifuge 18000g, 5min, 4°C .
 - Suck out PBS. Cells will be collected at the bottom.
 - place all tubes in -80°C freezer.

BioVision #106-100-1 Lot# 21151061

— Protein extraction

- 10x lysis buffer diluted 1:10 with dd ultrapure H₂O
- 1 tablet protease inhibitor added (Roche Diagnostics #11836153001)
- Add 400 μL 1x lysis buffer to each tube ($\sim 1 \times 10^7$ cells)
 - incubated 30 min
 - Centrifuge 13000 rpm, 10min, 4°C
 - transfer supernatant to new 1.5ml tube = Protein (-80°C)

Sample ID		
356	EL1 Unt	
357	EL1 5 ug/ml Talc	
358	EL1 20 ug/ml Talc	
359	EL1 100 ug/ml Talc	
360	SKOV-3 unt	
361	SKOV-3 5ug/ml	
362	SKOV-3 20ug/ml	
363	SKOV-3 100ug/ml	
364	TOV112 Unt	
365	TOV112 5 ug/ml Talc	
366	TOV112 20 ug/ml Talc	
367	TOV112 100 ug/ml Talc	
368	A2780 Unt	
369	A2780 5 ug/ml	
370	A2780 20 ug/ml	
371	A2780 100 ug/ml	
379	FT33 unt	
380	FT33 5ug/ml	
381	FT33 20 ug/ml	
382	FT33 100 ug/ml	
383	NOE unt	
384	NOE 5 ug/ml Talc	
385	NOE 20 ug/ml Talc	
386	NOE 100 ug/ml Talc	

1/8/2018

BCA protein detection Assay

(Pierce cat # 23225)

- Samples ID see pg 54

$$\begin{array}{c} \uparrow \\ \text{samples} \end{array} (24 \times 3 + 3 \text{ extra} + 3 \text{ blank}) = 78 \begin{array}{c} \uparrow \\ \text{wells} \end{array}$$

$$200 \mu\text{L per Well} = 200 \mu\text{L} \times 78 = 15600 \mu\text{L}$$

• 1 mL Reagent B per 50 mL Reagent A

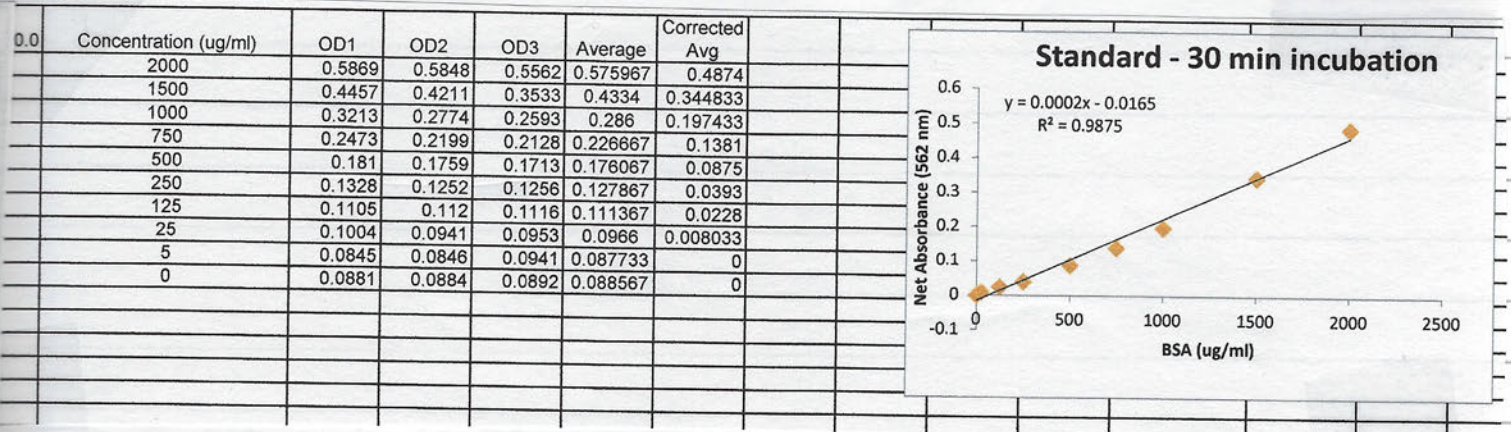
$$\frac{15600}{50} = 312 \mu\text{L}$$

• 15600 μL Reagent A + 312 μL Reagent B

- Assay

- Add 10 μL Sample to 3 wells
- Add 10 μL of blank to 3 wells (whatever you lysed your cells with)
- Add 200 μL of mix to each well
- Mix, incubate at 37°C 30 minutes
- * let plate to reach room temp
- Read at 562 nm with spectrophotometer

- Stand Curve



Compare results, with blank subtracted. to the standard curve

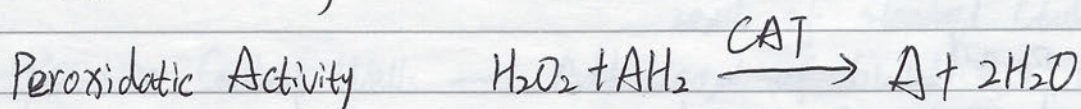
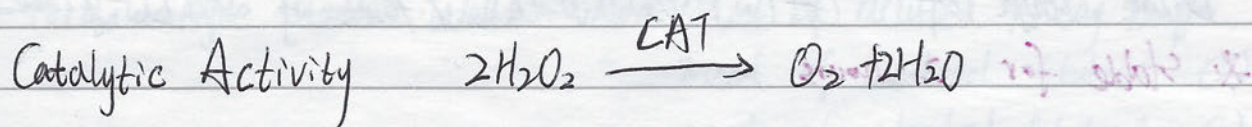
Compare results, with Blanks subtracted,
To the standard curve which has been previously determined

30 minute incubation										
ID	OD1	OD2	OD3	- blank 1	- blank 2	-blank 3	ug/ml 1	ug/ml 2	ug/ml 3	Average (mg/ml)
TOV-112-C	0.2599	0.2418	0.223	0.1713	0.1532	0.1344	939.1667	848.6667	754.6667	0.8475
TOV112-5 ug	0.3313	0.3057	0.2243	0.2427	0.2171	0.1357	1296.167	1168.167	761.1667	1.232167
TOV112- 20ug	0.1986	0.1784	0.1741	0.11	0.0898	0.0855	632.6667	531.6667	510.1667	0.520917
TOV112-100 ug	0.4219	0.3751	0.3853	0.3333	0.2865	0.2967	1749.167	1515.167	1566.167	1.540667
SKOV-3-C	0.5228	0.5485	0.4355	0.4342	0.4599	0.3469	2253.667	2382.167	1817.167	2.317917
SKOV-3-5 ug	0.3486	0.2963	0.2995	0.26	0.2077	0.2109	1382.667	1121.167	1137.167	1.129167
SKOV-3-20 ug	0.5041	0.5503	0.4834	0.4155	0.4617	0.3948	2160.167	2391.167	2056.667	2.202667
SKOV-3-100 ug	0.5336	0.5384	0.511	0.445	0.4498	0.4224	2307.667	2331.667	2194.667	2.278
A2780-C	0.5125	0.5118	0.5274	0.4239	0.4232	0.4388	2202.167	2198.667	2276.667	2.200417
A2780-5 ug	0.5112	0.5135	0.5888	0.4226	0.4249	0.5002	2195.667	2207.167	2583.667	2.201417
A2780-20 ug	0.5432	0.5026	0.517	0.4546	0.414	0.4284	2355.667	2152.667	2224.667	2.188667
A2780-100 ug	0.5229	0.4448	0.377	0.4343	0.3562	0.2884	2254.167	1863.667	1524.667	1.880833
Normal ovarian-C	0.3136	0.2745	0.2506	0.225	0.1859	0.162	1207.667	1012.167	892.6667	1.0375
Normal Ovarian-Talc 5 ug	0.4511	0.4449	0.4128	0.3625	0.3563	0.3242	1895.167	1864.167	1703.667	1.821
Normal ovarian- Talc 20 ug	0.553	0.5402	0.5244	0.4644	0.4516	0.4358	2404.667	2340.667	2261.667	2.335667
Normal Ovarian-100 ug	0.4285	0.4308	0.4289	0.3399	0.3422	0.3403	1782.167	1793.667	1784.167	1.786667
Fallopian-C	0.3884	0.373	0.373	0.2998	0.2844	0.2844	1581.667	1504.667	1504.667	1.530333
Fallopian-5 ug	0.4075	0.4286	0.4376	0.3189	0.34	0.349	1677.167	1782.667	1827.667	1.7625
Fallopian-20ug	0.6752	0.67	0.6842	0.5866	0.5814	0.5956	3015.667	2989.667	3060.667	3.022
Fallopian-100 ug	0.2599	0.2418	0.223	0.1713	0.1532	0.1344	939.1667	848.6667	754.6667	0.8475
EL-1-C	0.5268	0.4749	0.4474	0.4382	0.3863	0.3588	2273.667	2014.167	1876.667	2.054833
EL-1-5 ug	0.269	0.2655	0.2811	0.1804	0.1769	0.1925	984.6667	967.1667	1045.167	0.999
EL-1-20 ug	0.5264	0.5212	0.5391	0.4378	0.4326	0.4505	2271.667	2245.667	2335.167	2.284167
EL-1-100 ug	0.5438	0.5555	0.5387	0.4552	0.4669	0.4501	2358.667	2417.167	2333.167	2.369667

1/11/2018

Catalase ELISA

Cayman chem. Cat # 707002



- Assay uses peroxidatic activity to determine enzymatic activity.
- The enzyme with methanol in presence of optimal H_2O_2
 - The formaldehyde produced is measured colorimetrically with Purpald

Preparation

① - CAT assay buffer:

- dilute 2ml of buffer concentrate with 18ml HPLC-grade water
- Store at 4°C , for 2 months

② - CAT sample buffer

- dilute 5ml buffer with 45ml HPLC-grade water
- Use to dilute the formaldehyde standards, control, samples
- Store at 4°C , 2 months

③ - CAT Formaldehyde Standard

- The vial contains ~~4.5~~ 4.25 M formaldehyde
- Ready to use

④ - CAT (control)

Add 2ml of diluted Sample Buffer

Further dilute by taking 100µl + 1.9ml sample buffer

* Only stable for 30min

* Reconstituted CAT (control) is stable for one month at -20°C

⑤ - CAT Potassium Hydroxide

Add 4ml of 10µM KOH.

- Stable 3month at 4°C

⑥ - CAT Hydrogen Peroxide

Dilute 40 μ l of CAT H₂O₂ with 9.96ml of HPLC-grade H₂O
 * Stable for 2 hours

⑦ - CAT Purpald

Contains 4ml of purpald in 0.5M hydrochloric acid
 Ready to use

⑧ - CAT Potassium Periodate

Contains 1.5ml of potassium Periodate in 0.5 M potassium hydroxide

- STANDARD

- Dilute 10 μ l of CAT Formaldehyde standard with 9.99ml of diluted Sample Buffer to obtain a 4.25 mM formaldehyde stock solution
- Label tubes A - G, add accordingly

Plate Set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	1	362	1	264	1	579	1				
B	B	1	369	1	365	1	380	1				
C	C	1	370	1	366	1	381	1				
D	D	1	371	1	367	1	382	1				
E	E	1	360	1	383	1	356	1				
F	F	1	361	1	384	1	357	1				
G	G	1	362	1	385	1	358	1				
H	+	+	363	1	386	1	359	1				

Tube	Formaldehyde (μ l)	Sample Buffer (μ l)	Final (μ l)
A	0	1,000	
B	10	990	
C	30	970	
D	60	940	
E	90	910	
F	120	880	
G	150	850	

* Final Formaldehyde concentration in the 170 μ l reaction

A-G = standards

+

51-510 = samples well

- Performing the Assay

- Formaldehyde Standard Wells - Add 100 μ l of diluted Assay Buffer.
 - 30 μ l of methanol (tubes A - G)
 - 20 μ l of standard (tubes A - G)
- Positive Control Wells - Add 100 μ l of diluted assay buffer
 - 30 μ l of methanol
 - 20 μ l of diluted Catalase Control
- Sample well Add 100 μ l of diluted Assay buffer
 - 30 μ l of methanol
 - 20 μ l of sample to two well

- Start Reaction by adding 20 μ l of diluted Hydrogen Peroxide
* Note start time. Wave fast

- Cover plate, incubate on shaker 20 minutes. Room temperature

- Add 30 μ l of Potassium Hydroxide to each well to terminate Reaction

- add 30 μ l CAT purpal to each well
- Cover plate, incubator for 10 minutes on shaker. Room temp.

- Add 10 μ l CAT Potassium Periodate

- Cover plate. 5 minutes, shaker. Room temp.

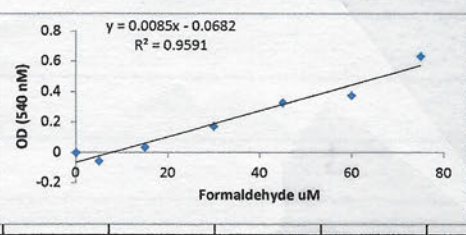
- Read the absorbance at 540

- Assay sensitive between 2 ~ 35 nmol/min/ml
- Catalase positive controls should give you absorbance ~ 0.29

— Calculation

- Calculate the average absorbances of each standard and samples
- Subtract the average of OD_{standard A} from itself and all other standard. samples
- Plot corrected absorbance of standards (y-axis) VS formaldehyde concentration (μM) from equation obtained from standard curve

Standard	OD 1 (540 nm)	OD 2 (540 nm)	Average	Corrected Av	Formaldehyde (μM)
A	0.1312	0.1502	0.1407	0	0
B	0.1863	0.1786	0.18245	-0.0584167	5
C	0.2813	0.2705	0.2759	0.0350333	15
D	0.3882	0.4365	0.41235	0.1714833	30
E	0.5317	0.6039	0.5678	0.3269333	45
F	0.5171	0.7139	0.6155	0.3746333	60
G	0.903	0.8398	0.8714	0.6305333	75
Positive Control	0.858	0.7262	0.7921	0.5512333	



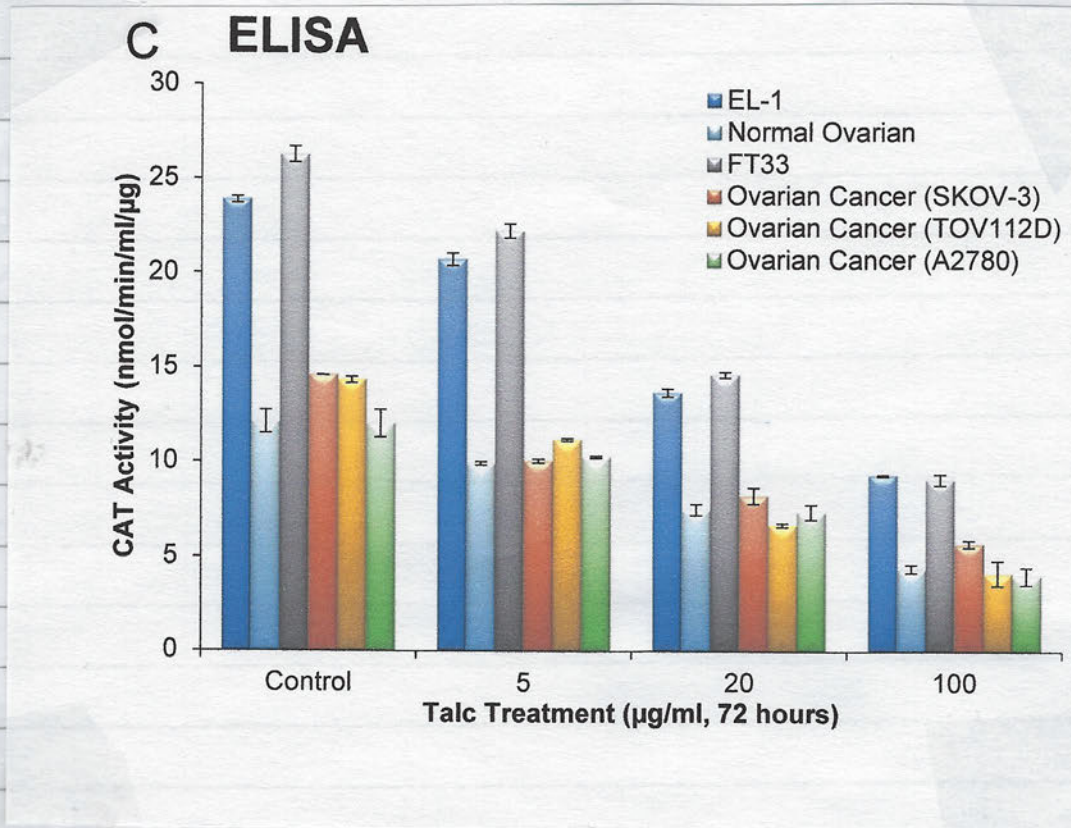
- Calculate the formaldehyde concentration of the samples using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each samples

$$\text{Formaldehyde } (\mu\text{M}) = \left[\frac{\text{Sample absorbance} - (y\text{-intercept})}{\text{slope}} \right] \times \frac{0.1 \text{ ml}}{0.02 \text{ ml}}$$

- Calculate the CAT activity of the sample using the following equation. One unit is defined as the amount of enzyme will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C

$$\text{CAT activity} = \frac{\text{nmol of sample}}{20 \text{ min}} \times \text{Sample dilution} = \text{nmol/min/ml}$$

		OD 1	OD 2	OD 3	Corr 1	Corr2	Corr3	uM 1	uM 2	uM 3	nmol/min/ml	nmol/min/ml	nmol/min/ml	nmol/min/ml	2 ug protein used	SD
1/11/2018	BLANK	0.2598	0.2361	0.2267	0.24086667										Average	
	A2780-C	0.3723	0.4053	0.3827	0.1314333	0.1644333	0.1418333	199.63333	232.63333	210.03333	9.98	11.63	10.50	11.07		0.80
	A2780-5 ug	0.3562	0.3855	0.3544	0.1153333	0.1446333	0.1155333	183.53333	212.83333	181.73333	9.18	10.64	9.09	9.13		0.06
	A2780-20 ug	0.2978	0.287	0.2857	0.0569333	0.0461333	0.0448333	125.13333	114.33333	113.03333	6.26	5.72	5.65	5.95		0.43
	A2780-100 ug	0.2153	0.211	0.225	-0.0255667	-0.0298667	-0.0158667	42.63333	38.33333	52.33333	2.13	1.92	2.62	2.27		0.49
	SKOV-3-C	0.4022	0.4504	0.4506	0.1613333	0.2095333	0.2097333	229.53333	277.73333	277.93333	11.48	13.89	13.90	13.89		0.01
	SKOV-3-5 ug	0.3839	0.3521	0.3489	0.1430333	0.1112333	0.1080333	211.23333	179.43333	176.23333	10.56	8.97	8.81	8.89		0.11
	SKOV-3-20 ug	0.3168	0.3037	0.3438	0.0759333	0.0628333	0.1029333	144.13333	131.03333	171.13333	7.21	6.55	8.56	6.88		0.46
	SKOV-3-100 ug	0.2118	0.2988	0.2177	-0.0290667	0.0579333	-0.0231667	39.13333	126.13333	45.03333	1.96	6.31	2.25	4.13		3.08
	TOV-112-C	0.4422	0.4475	0.4411	0.2013333	0.2066333	0.2002333	269.53333	274.83333	268.43333	13.48	13.74	13.42	13.61		0.19
	TOV112-5 ug	0.3742	0.3762	0.3737	0.1333333	0.1353333	0.1328433	201.53333	203.53333	201.04333	10.08	10.18	10.05	10.13		0.07
	TOV112-20ug	0.2783	0.27531	0.26998	0.0374333	0.0344433	0.0291133	105.63333	102.64333	97.313333	5.28	5.13	4.87	5.21		0.11
	TOV112-100 ug	0.211	0.2314	0.2251	-0.0298667	-0.0094667	-0.0157667	38.33333	58.73333	52.43333	1.92	2.94	2.62	2.43		0.72
	Normal ovarian-C	0.3862	0.4055	0.3934	0.1453333	0.1646333	0.1525333	213.53333	232.83333	220.73333	10.68	11.64	11.04	11.16		0.68
	Normal Ovarian-															
	Taic 5 ug	0.3492	0.3461	0.3444	0.1083333	0.1052333	0.1035333	176.53333	173.43333	171.73333	8.83	8.67	8.59	8.75		0.11
	Normal ovarian-															
	Taic 20 ug	0.2987	0.28972	0.28702	0.0578333	0.0488533	0.0461533	126.03333	117.05333	114.35333	6.30	5.85	5.72	6.08		0.32
	Normal Ovarian-															
	100 ug	0.2231	0.2298	0.23111	-0.0177667	-0.0110667	-0.0097567	50.43333	57.13333	58.44333	2.52	2.86	2.92	2.69		0.24
	Fallopian-C	0.7118	0.6588	0.6177	0.4709333	0.4579333	0.3768333	539.13333	526.13333	445.03333	26.96	26.31	22.25	26.63		0.46
	Fallopian-5 ug	0.6112	0.62301	0.6222	0.3703333	0.3821433	0.3813333	438.53333	450.34333	449.53333	21.93	22.52	22.48	22.22		0.42
	Fallopian-20ug	0.4534	0.4487	0.4222	0.2125333	0.2078333	0.1813333	280.73333	276.03333	249.53333	14.04	13.80	12.48	13.92		0.17
	Fallopian-100 ug	0.3245	0.3333	0.3198	0.0836333	0.0924333	0.0789333	151.83333	160.63333	147.13333	7.59	8.03	7.36	7.81		0.31
	EL-1-C	0.6554	0.6498	0.6511	0.4145333	0.4089333	0.4102333	482.73333	477.13333	478.43333	24.14	23.86	23.92	24.00		0.20
	EL-1-5 ug	0.5891	0.57991	0.5899	0.3482333	0.3380433	0.3490333	416.43333	406.24333	417.23333	20.82	20.31	20.86	20.57		0.36
	EL-1-20 ug	0.4332	0.4265	0.4544	0.1923333	0.1856333	0.2135333	260.53333	253.83333	281.73333	13.03	12.69	14.09	12.86		0.24
	EL-1-100 ug	0.3332	0.3345	0.3582	0.0923333	0.0936333	0.1173333	160.53333	161.83333	185.53333	8.03	8.09	9.28	8.06		0.05



1/17/2018

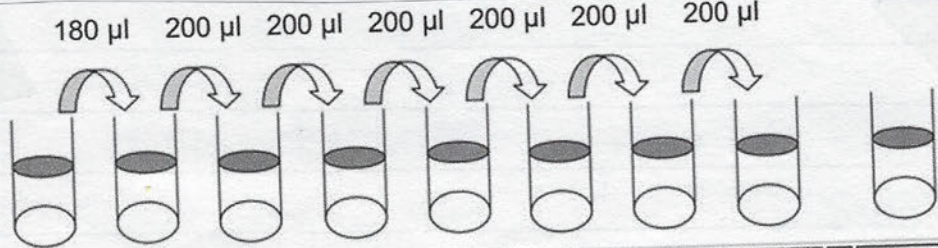
CA-125 ELISA

RayBio. Cat # ELH-CA125

- This assay employs an antibody specific for human CA-125 coated on 96-well plate.
- CA125 present in sample is bound to the wells
- Wash away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells,
- Wash again, color develop in proportion the amount of CA-125 bound
- Stop Solution, and measured at 450nm

- Preparation

- put all reagents and samples to room temperature (18-25°C).
- Assay Buffer Diluent diluted 5-fold with distilled H₂O.
- Sample dilution: 1X assay Diluent use for dilution of serum samples.
 - The suggested dilution for normal serum/plasma is 2 fold
 - * levels of CA-125 may vary between different samples.
 - Optimal dilution factors for each sample must be determined by the investigator
- Preparation of Standard: Briefly spin a vial of Item C.
 - ~~add~~ Use the 400 U/ml standard solution to produce a dilution series (see below)



		Std1	Std2	Std3	Std4	Std5	Std6	Std7	Zero Standard
Diluent volume	Item C+ 400 µl	270 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl
Conc.	1,000 U/ml	400 U/ml	133.3 U/ml	44.45 U/ml	14.81 U/ml	4.94 U/ml	1.65 U/ml	0.55 U/ml	0 U/ml

SAED000035(color)

- If the wash Concentrate (20x) contains visible crystals, warm to room temperature and mix gently.
 - Dilute 20ml of wash buffer concentrate into deionized or distilled water to yield 400ml of 1X Wash Buffer
- Briefly spin the Detection Antibody vial before use.
 - add 100µl of 1X assay diluent into the vial to prepare a detection antibody solution
* stored at 4°C for 5 days
- Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently.
 - diluted 800-fold with 1X Assay Diluent

— Assay Procedure

- Bring all reagents and samples to room temperature (18~25°C)
- Label removable 8-well strips as appropriate for your experiment
- Add 100µl of each standard and sample into appropriate wells
 - cover wells and incubate for 2.5 hours at room temp. gently shaking
- Discard the solution and wash 4 times with 1X Solution.
 - Wash with 300µl wash Buffer.
 - Complete removal of liquid
 - After the last wash, remove any remaining wash buffer by aspirating
- add 100µl of 1X prepared biotinylated antibody
 - Incubate for 1 hour at room temperature, gently shaking
- Discard the solution, Repeat the wash

- 100 μ l of prepared Streptavidin Solution to each well
 - Incubate for 45 minutes, room temperature with gently shaking
- Discard the solution. Repeat the wash
- Add 100 μ l of TMB One-Step Substrate Reagent
 - Incubate 30 mins, room temperature in dark, gently shaking
- Add 50 μ l of stop Solution to each well.
- Read at 450 nm immediately.

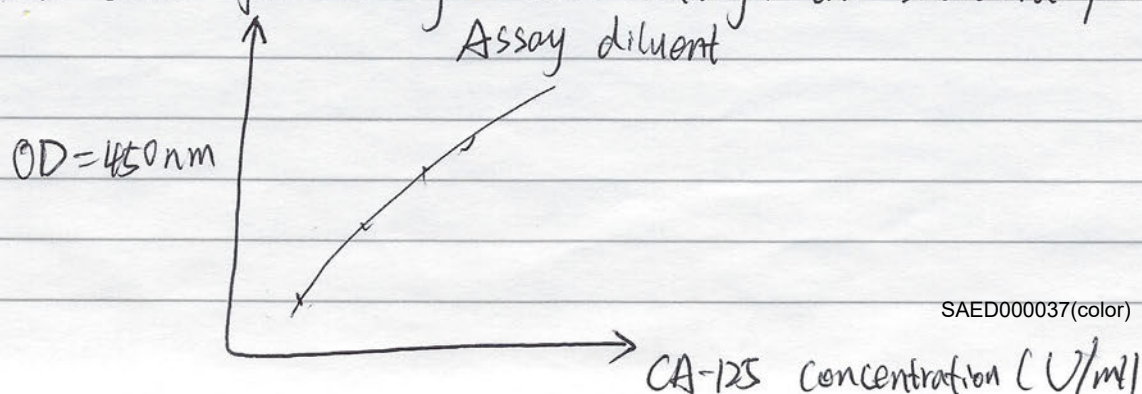
* The minimum detectable dose of CA-125 was determined to be 0.6 U/ml

* Intra-Assay CV%: < 10%

* Inter-Assay CV%: < 12%

— Calculation of results

- Calculate the mean absorbance for each set of duplicate standards, controls and samples and subtract the average zero standard optical density.
- Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis
- Draw the best-fit straight line through the standard points



— Recovery was determined by spiking various levels of CA-125 into the sample types listed below.

Sample Type	Average % Recovery	Range (%)
Serum	97.21	89-107
Plasma	76.88	68-85
Cell culture media	85.34	76-130

— Linearity

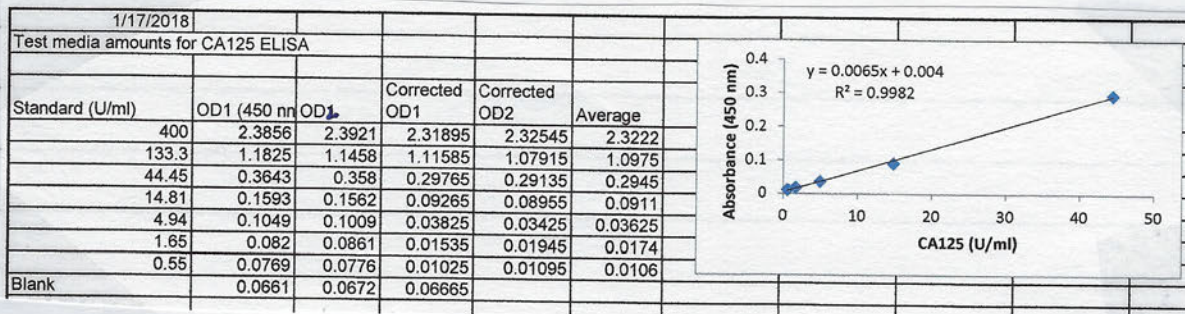
Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	110.0	130.2	135.9
	Range (%)	99-118	119-138	125-142
1:4	Average % of Expected	107.5	126.4	92.99
	Range (%)	96-116	117-135	83-103

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	1	Tau112	1	1	MOE	1					
B	B	1	Tau112 + Talc	1	1	MOE + Talc	1					
C	C	1	A2780	1	1	Skov-3	1					
D	D	1	A2780 + Talc	1	1	Skov-3 + Talc	1					
E	E	1	EL-1	1	1							
F	F	1	EL-1 + Talc	1	1							
G	G	1	FTI	1	1							
H	Blank	1	FTI + Talc	1	1							

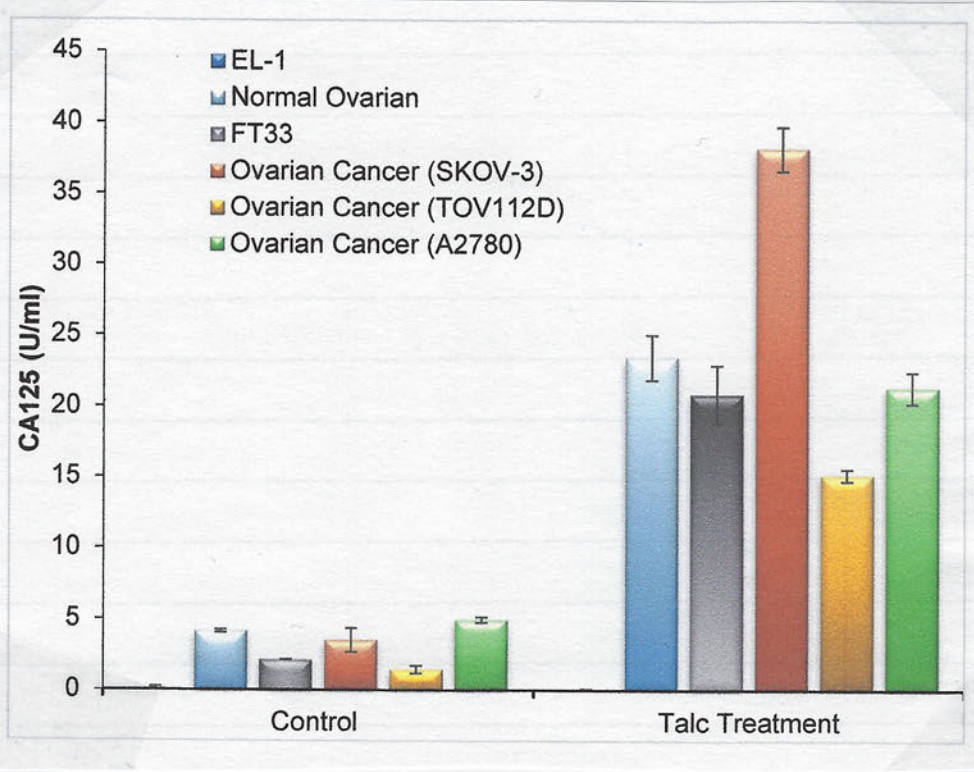
Plate set up

Talc Treatment: 100 µg/ml

Human CA-125 standard curve.



Test Media	0.0799	0.0885	0.0811	0.01325	0.02185	0.01445	0.01385						
OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	Corrected for Media OD1	Corrected for Media OD2	Corrected for Media OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	
V112	0.096	0.0925	0.0939	0.02935	0.02585	0.02725	0.0155	0.012	0.0134	1.769230769	1.230769231	1.446153846	1.482051282
V112+Talc	0.1849	0.1799	0.1843	0.11825	0.11325	0.11765	0.1044	0.0994	0.1038	15.44615385	14.67692308	15.35384615	15.15897436
780	0.1179	0.1155	0.1172	0.05125	0.04885	0.05055	0.0374	0.035	0.0367	5.138461538	4.769230769	5.030769231	4.979487179
780+ Talc	0.2216	0.2312	0.2172	0.15495	0.16455	0.15055	0.1411	0.1507	0.1367	21.09230769	22.56923077	20.41538462	21.35897436
1	0.0854	0.0862	0.0851	0.01875	0.01955	0.01845	0.0049	0.0057	0.0046	0.138461538	0.261538462	0.092307692	0.164102564
1+ Talc	0.0779	0.0795	0.0972	0.01125	0.01285	0.03055	-0.0026	-0.001	0.0167	-1.01538462	-0.76923077	1.953846154	0.056410256
lopian	0.0988	0.0985	0.0985	0.03215	0.03185	0.03185	0.0183	0.018	0.018	2.2	2.153846154	2.153846154	2.169230769
lopian + Talc	0.2112	0.2355	0.2144	0.14455	0.16885	0.14775	0.1307	0.155	0.1339	19.49230769	23.23076923	19.98461538	20.9025641
mal Ovarian	0.1111	0.1124	0.1114	0.04445	0.04575	0.04475	0.0306	0.0319	0.0309	4.092307692	4.292307692	4.138461538	4.174358974
mal ovarian + Talc	0.222	0.234	0.255	0.15535	0.16735	0.18835	0.1415	0.1535	0.1745	21.15384615	23	26.23076923	23.46153846
OV-3	0.1012	0.1103	0.1111	0.03455	0.04365	0.04445	0.0207	0.0298	0.0306	2.569230769	3.969230769	4.092307692	3.543589744
OV3 +talc	0.3389	0.3211	0.3384	0.27225	0.25445	0.27175	0.2584	0.2406	0.2579	39.13846154	36.4	39.06153846	38.2

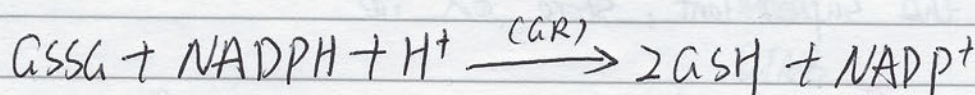
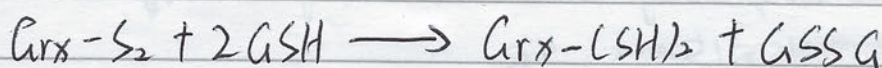
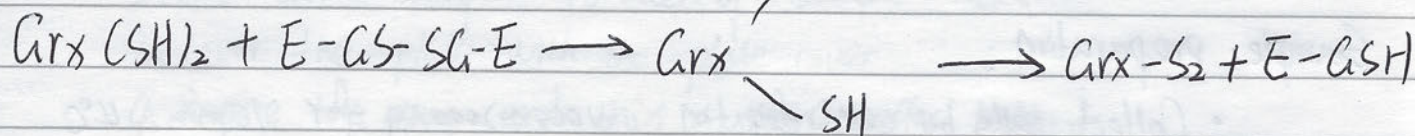


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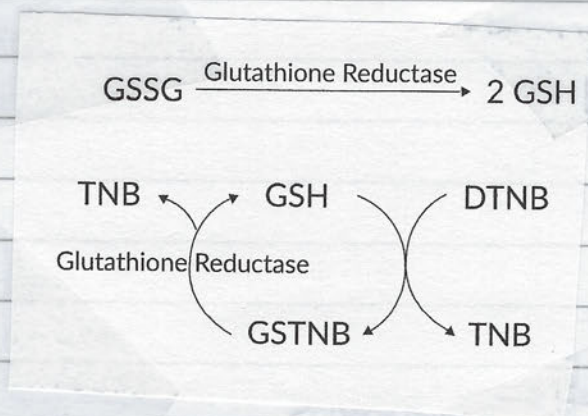
Glutathione assay

(Cayman chemical Cat # 703002)

S-SG-E



- This kit measure the amount of total glutathione (GSH + GSSG)
- GSH is easily oxidized to the disulfide dimer GSSG. GSSG is produced during the reduction of hydroperoxides by glutathione peroxidase.
- This kit can also be used to measure only GSSG



GSH recycling

Reagent Preparation

- GSH MES Buffer (2x): 0.4M 2-ethanesulphonic acid, 0.1M phosphate, 2mM EDTA
- Dilute 60mL of buffer with 60mL of HPLC-water
- GSSG standard: 2mL of 25mM GSSG in MES buffer
- Ready to use
- GSH Co-Factor Mixture: a lyophilized powder of NADP⁺ and glucose-6-phosphate
- add 0.5mL HPLC-water
- GSH Enzyme mixture: glutathione reductase and glucose-6-phosphate in 0.2mL Buffer
- add 2mL of diluted MES Buffer

— GSH DTNB : a lyophilized powder of DTNB

Sample preparation:

- Collect cells by centrifugation, 1000~2000xg for 10min, 4°C
- The cell pellet can be homogenized in 1-2ml of cold buffer.
- Centrifuge 10000xg, 15min, 4°C
- Remove the supernatant, store on ice

Assay protocol:

— plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	1	383	1	356	1	360	1				
B	B	1	384	1	357	1	361	1				
C	C	1	385	1	358	1	362	1				
D	D	1	386	1	359	1	363	1				
E	E	1	379	1	368	1	364	1				
F	F	1	380	1	369	1	365	1				
G	G	1	381	1	370	1	366	1				
H	H	1	382	1	371	1	367	1				

Standards

Standard preparation

- Take eight clean test tubes and mark them A-H
- Aliquot the GSSG standard and MES buffer to each tube as described in table.

Tube	GSSG Standard (μl)	MES Buffer (μl)	Final Concentration (μM GSSG)	Equivalent Total GSH (μM)*
A	0	500	0	0
B	5	495	0.25	0.5
C	10	490	0.5	1.0
D	20	480	1.0	2.0
E	40	460	2.0	4.0
F	80	420	4.0	8.0
G	120	380	6.0	12.0
H	160	340	8.0	16.0

SAED000042(color)

Performing the Assay

1. Add 50μl Standard (A-H)
2. Add 50μl samples to each of sample wells
3. Cover the plate with the plate cover
4. Prepare the assay, and mix:

MES buffer	11.25ml
Cofactor mixture	0.45ml
Enzyme mixture	2.1ml
Water	2.3 ml
DTNB	0.45ml
5. Remove the plate cover and add 150μl of freshly prepared Assay Cocktail to each of wells.
 - Replace the plate cover.
 - Incubate the plate in the dark on an orbital shaker
6. GSH concentration of samples determined by the End Point Method.
 - End point Method: Read Plate at 405-414nm after 25 minutes
 - Kinetic Method: Read the plate at 405-414nm at 5 minutes intervals for 30 minutes.

Analysis

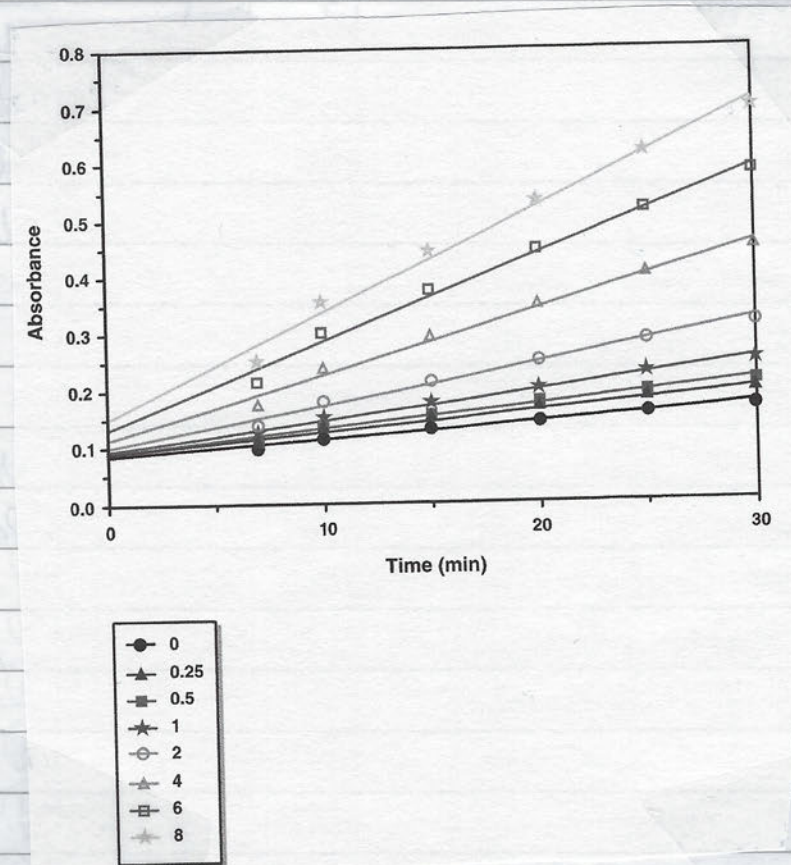
1. calculate the average absorbance from 25 minutes for each standard and sample
2. Subtract the absorbance value of the standard A from itself and all other values.
3. Plot the corrected absorbance values of each standard as a function of the concentration of ASSA or Total GSH
4. Calculate the values of ASSA or Total GSH for each sample from the standard curve

$$\text{Total GSH or ASSA} = \frac{A_{405} - y\text{-intercept}}{\text{slope}} \times 2 \times \text{Sample dilution}$$

If sample required deprotection multiply by "2" to account for the addition of MPA Reagent

Kinetic Method

1. Plot the average absorbance values of each standard and sample as a function of time and determine the slope for each curve



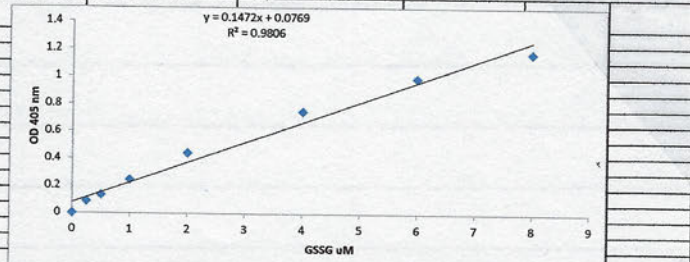
Plot of absorbance versus time for each standard

2. Plot the i-slopes of each standard as a function of concentration of ASSA
3. Calculate the values of ASSA for each sample from their respective slopes using the slope versus ASSA standard curve.

$$\text{Total GSH or ASSA} = \frac{(\text{i-slope for sample}) - \text{y-intercept}}{\text{f-slope}} \times 2 \times \text{sample dilution}$$

* Inter-assay coefficient of variation is 3.5%
 * Inter-assay coefficient is 1.6%

Standard GSSG uM	OD1	OD2	OD3	Average	Corrected
0	0.2398	0.2422	0.2396	0.240533333	0
0.25	0.3139	0.3119	0.3532	0.326333333	0.0858
0.5	0.3769	0.3713	0.3738	0.374	0.133466667
1	0.4877	0.4856	0.4845	0.485933333	0.2454
2	0.6801	0.6853	0.6807	0.682033333	0.4415
4	0.9867	0.99	0.9868	0.987833333	0.7473
6	1.2273	1.2338	1.2322	1.2311	0.990566667
8	1.4006	1.4119	1.4267	1.413066667	1.172533333



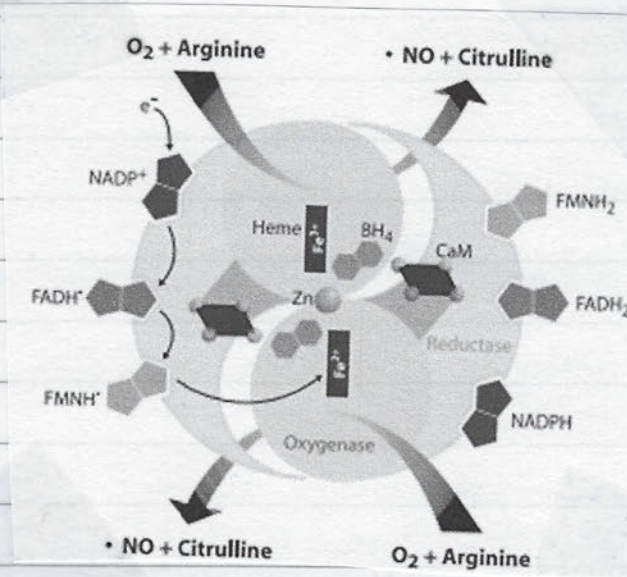
2/20/2018 using 30ug protein												
SAMPLE	OD1	OD2	OD3	uM GSSG	uM GSSG	uM GSSG	DF	x DF x2 for deprot	x DF x2 for deprot	x DF x2 for deprot	Average	SD
Normal ovarian-C	0.8465	0.9063	0.9107	0.605966667	0.665766667	0.670166667	2.6984127	3.270296296	3.593026455	3.616772487	3.6048995	0.016791
Normal ovarian-Talc 5 ug	0.768	0.7602	0.7945	0.527466667	0.519666667	0.553966667	17.989418	2.846645503	2.804550265	2.989661376	2.8802857	0.0970324
Normal ovarian-Talc 20 ug	0.5931	0.589	0.5346	0.352566667	0.348466667	0.294066667	5.3968254	1.902740741	1.880613757	1.587026455	1.8916772	0.0156461
Normal ovarian-100 ug	0.375	0.3655	0.3628	0.134466667	0.124966667	0.122266667	6.3968254	0.725693122	0.67442328	0.659851852	0.7000582	0.0362533
Fallopian-C	1.2553	1.2725	1.2698	1.014766667	1.031966667	1.029266667	7.3968254	5.476518519	5.569343915	5.554772487	5.5229312	0.0656375
Fallopian-5 ug	0.9852	0.9655	0.9746	0.744666667	0.724966667	0.734066667	8.3968254	4.018835979	3.912518519	3.96162963	3.9656772	0.0751778
Fallopian-20ug	0.7626	0.7666	0.7584	0.522066667	0.526066667	0.517866667	9.3968254	2.817502646	2.839089947	2.794835979	2.8282963	0.0152645
Fallopian-100 ug	0.4561	0.4121	0.4872	0.215566667	0.171566667	0.246666667	10.3968254	1.163375661	0.925915344	1.331216931	1.0446455	0.1679098
EL-1-C	1.1861	1.0981	1.2	0.945566667	0.857566667	0.959466667	12.3968254	5.103058201	4.628137566	5.178074074	4.8655979	0.3358196
EL-1-5 ug	0.9011	0.8911	0.911	0.660566667	0.650566667	0.670466667	13.3968254	3.564962963	3.510994709	3.618391534	3.5379788	0.0381613
EL-1-20 ug	0.711	0.871	0.8812	0.470466667	0.630466667	0.640666667	14.3968254	2.539026455	3.402518519	3.457566138	2.9707725	0.6105811
EL-1-100 ug	0.4555	0.544	0.511	0.214966667	0.303466667	0.270466667	15.3968254	1.160137566	1.637756614	1.459661376	1.3989471	0.3377277
A2780-C	1.366	1.411	1.366	1.125466667	1.170466667	1.125466667	17.3968254	6.07394709	6.316804233	6.07394709	6.1953757	0.1717259
A2780-5 ug	1.244	1.1482	1.122	1.003466667	0.907666667	0.881466667	18.3968254	5.415534392	4.898518519	4.757121693	5.1570265	0.3655854
A2780-20	0.9551	0.8792	0.799	0.714566667	0.638666667	0.558466667	19.3968254	3.856391534	3.446772487	3.01394709	3.651582	0.2896444
SKOV-3-C	0.5111	0.5514	0.6331	0.270566667	0.310866667	0.392566667	20.3968254	1.460201058	1.677693122	2.118613757	1.5689471	0.1537901
SKOV-3-5	1.2297	1.311	1.295	0.989166667	1.070466667	1.054666667	21.3968254	5.338359788	5.777121693	5.690772487	5.5577407	0.3102515
SKOV-3-20	1.0221	1.1087	1.098	0.781566667	0.868166667	0.857466667	22.3968254	4.217978836	4.685343915	4.627597884	4.4516614	0.330477
SKOV-3-100	0.8972	0.9112	0.799	0.656666667	0.670666667	0.558466667	23.3968254	3.543915344	3.619470899	3.01394709	3.5816931	0.0534258
TOV-112-C	0.555	0.6113	0.599	0.314466667	0.370766667	0.358466667	24.3968254	1.697121693	2.000962963	1.934582011	1.8490423	0.2148482
TOV112-5	1.1027	1.226	1.301	0.862166667	0.985466667	1.060466667	25.3968254	4.652962963	5.318391534	4.9856772	4.9856772	0.4705291
TOV112-20ug	0.8932	0.9032	0.991	0.652666667	0.662666667	0.750466667	26.3968254	3.522328042	3.576296296	4.050137566	3.5493122	0.0381613
TOV112-100	0.6671	0.5982	0.555	0.426566667	0.357666667	0.314466667	27.3968254	2.30210582	1.93026455	1.697121693	2.1161852	0.2629315
ug	0.444	0.3897	0.3775	0.203466667	0.149166667	0.136966667	28.3968254	1.098074074	0.805026455	0.739185185	0.9515503	0.207216

Nitrate/Nitrite Assay Kit (LDH method)

2/25/2018

Cat # 760871

- Nitric Oxide (NO) is synthesized in biological systems by the Nitric Oxide Synthase (NOS)
- NOS is remarkably complex enzyme which acts on molecular oxygen, arginine, and NADPH to produce NO, citrulline, and NADP⁺
- This process requires five additional cofactors and two divalent cations.



Nitric Oxide Synthesis

Nitric Oxide Synthase Isoforms

